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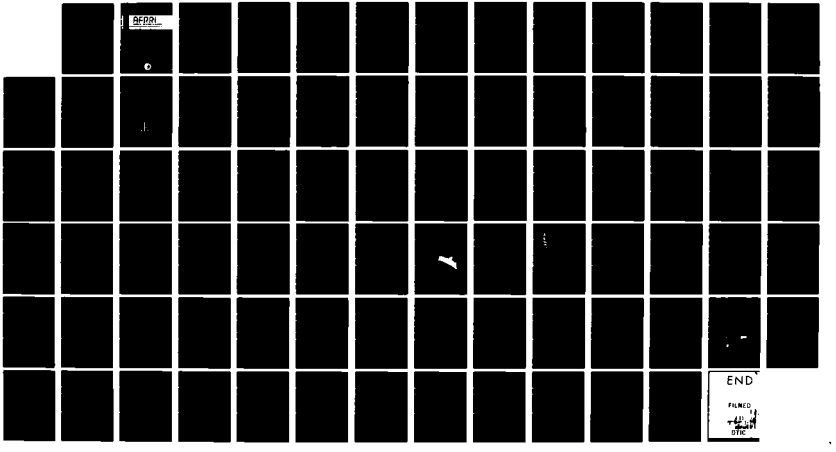

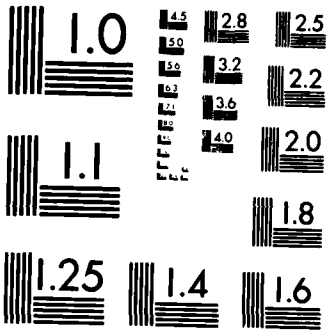

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Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council.

Studies involving human patients were performed in conformity with the "recommendations guiding doctors in clinical research" as stated in the Declaration of Helsinki of the World Health Medical Association (1964).

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This report contains a summary of the research projects of the Armed Forces Radiobiology Research Institute for the period 1 October 1978 through 30 September 1979.

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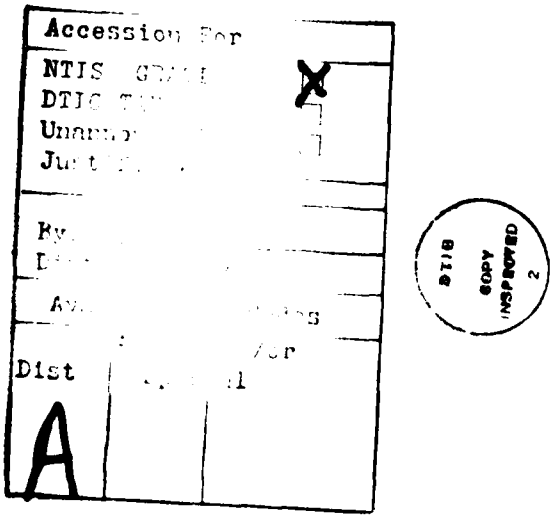

BEHAVIORAL SCIENCES DEPARTMENT

The Behavioral Sciences Department is engaged in research to determine the acute effects of radiation, chemicals, and drugs on the behavior, performance, psychoneurological integrity, and physiology of experimental animals, for extrapolation of these data to man. The chemicals and drugs incorporated in research protocols are unique to military working environments. Research projects in support of this effort are conducted in two Divisions of the Department: the Experimental Psychology Division and the Physiological Psychology Division. Collaborative efforts exist with other departments of the Institute as well as with the Naval Medical Research Institute, the National Institutes of Health, and several area universities.

The research efforts of the Department incorporate a variety of animal models in order to assess the capability of man to function in environments involving tactical nuclear weapons. This assessment includes evaluation of complex mental and/or physical tasks, bioelectric and biochemical changes related to ionizing radiation exposure, and the development of data on possible methods to prevent or modify performance decrements caused by exposure to ionizing radiation.

Behavioral toxicology studies have also been developed and evaluated to detect changes in the neurophysiological and performance capability of primates and rodents. These tests provide information for establishing maximum permissible occupational levels for industrial and military environments, and have been developed for use in radiation-injury studies to detect subtle changes in behavior.

Results of the Department's research programs are forwarded to the military services and appropriate agencies by informal reports and incorporation into committee and working-group reports, discussion, and correspondence. They are also made available to the scientific community through publications and oral presentations at scientific meetings.

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EFFECTS OF PETROLEUM DFM AND JP5 ON BEHAVIOR OF RATS

Principal Investigators: V. Bogo and R. W. Young
Technical Assistance: G. G. Kessell and C. A. Boward

As part of Project Independence, the U.S. Navy is actively assessing alternatives to petroleum-derived fuels such as diesel fuel marine (DFM) and jet propulsion fuel number 5 (JP5) to determine the feasibility of extracting and refining these fuels from shale. Since no baseline information exists on the petroleum fuels, as part of an overall evaluation, preliminary work has been conducted for fuel-related health effects. To follow up findings of earlier work on acute oral petroleum, three specific behavioral evaluations were conducted with rats.

In the first study, rats were given either 1, 3, or 5 ml/kg of JP5 (per os) and observed at 30-min intervals for 6 hours to determine the level and temporal pattern of spontaneous activity. These observations were conducted during the day, when rats are normally dormant, in order to determine whether dormant-cycle activity would be affected by JP5 as had been observed previously for the active-cycle period. The results of this study indicated a significant increase in dormant-cycle activity at 2-1/2 hours after dosing in the 3-ml and 5-ml/kg groups, which lasted through the final testing period. However, the activity of the 1-ml/kg group was not affected during the 6 hours of testing. Besides setting the time to effect, this finding confirms the earlier observations of increased spontaneous overnight activity.

In the second study, rats were given 3 ml/kg of JP5 (per os) and tested at 30-min intervals on a trained motor integration task (accelerod) to determine if the increase in overall activity previously seen would also affect motor performance. Motor integration, as tested here, was not affected during the 6 hours of assessment. Thus, the previously observed elevated levels of spontaneous activity are not reflected in altered performance of motor integration.

The third study evaluated the effects of DFM on motor integration in exactly the same manner as the second study. The results of this study indicate that integrated motor performance decreased significantly at 2-1/2 hours after dosing. Our previous work demonstrated that oral DFM markedly depressed spontaneous activity. Thus it appears that DFM is capable of producing depression in both overall activity and integrated motor function.

Taken together, these three studies corroborate our previous observations that JP5 and DFM produce opposite overall effects on general activity, and fix the time of maximum effect for both materials at 2-1/2 hours after dosing. DFM affected integrated motor performance; JP5 did not. These findings suggest that these two materials act in different ways and thus present different potential problems to fleet safety and performance. DFM could produce depression in level of alertness and could potentially affect the ability of operational personnel to perform skilled combat tasks. JP5, on the other hand, does not seem to interfere with task capability, but does present a potential problem in terms of confused judgment or heightened irritability, as observed for other stimulant toxins. Both

the DFM and JP5 effects observed here could seriously impair performance of attention-demanding tasks. The observations are tentative, but it is noteworthy that the results were produced by single doses, which did not produce other signs of frank toxicity.

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BIOCHEMICAL STUDIES OF ACUTE AND CHRONIC INSULTS TO THE CENTRAL NERVOUS SYSTEM

Principal Investigator: W. A. Hunt
Technical Assistance: T. K. Dalton

Comparing the effects of radiation with insults of better-understood mechanisms of action can be quite helpful in understanding how ionizing radiation degrades behavior. For example, a number of drugs produce behavioral effects similar to those induced by ionizing radiation. The study of these drugs has provided important insights into how radiation causes its effects.

One drug of particular value in these studies is ethanol. Ethanol is a depressant that induces motor decrement and performance degradation, as does radiation. Neurochemically, ethanol affects two of the neurotransmitters in the basal ganglia, an area of the brain involved in motor coordination. Both dopaminergic activation and cholinergic activation in the caudate nucleus can be observed after ethanol treatment, but only when ethanol is present in the blood in animals administered ethanol (1,2). Added in vitro to the preparations, ethanol has no effect.

With this in mind, experiments were conducted to determine if similar effects could be observed after doses of ionizing radiation delivered from a linear accelerator. A 10-krad dose of high-energy electrons induced a transient increase in striatal dopamine release that correlated with the time-course of early transient incapacitation (ETI). High-affinity choline uptake, an index of acetylcholine release, was also elevated during the same period (3).

Since the response obtained after irradiation was similar to the response observed after treatment with dopaminergic antagonist, it was possible that the radiation in some way disrupted the ability of dopamine to interact with its receptor. Dopamine-sensitive adenylate cyclase activity and haloperidol binding, two indexes of dopaminergic receptor function, were determined after irradiation. No alterations were observed in either (3). Ethanol administration also failed to alter dopaminergic receptors (4).

In other experiments involving the cyclic nucleotides, the chronic reduction in cyclic GMP in brain induced by chronic ethanol treatment (5) suggested that an altered responsivity in the receptor for cyclic GMP might result. The activities of cyclic GMP-dependent protein kinase and an endogenous inhibitor of the

protein kinase were measured after chronic ethanol treatment. No changes in activity were observed. The effect of ionizing radiation on cyclic GMP and cyclic AMP levels in various areas of the brain is currently under investigation.

REFERENCES

1. Darden, J. H. and Hunt, W. A. Reduction of striatal dopamine release during an ethanol withdrawal syndrome. Journal of Neurochemistry 29: 1143-1145, 1977.

2. Hunt, W. A., Majchrowicz, E., and Dalton, T. K. Alterations in high-affinity choline uptake in brain after acute and chronic ethanol treatment. Journal of Pharmacology and Experimental Therapeutics 210: 259-263, 1979.

3. Hunt, W. A., Dalton, T. K., and Darden, J. H. Transient alterations in neurotransmitter activity in the caudate nucleus of rat brain after a high dose of ionizing radiation. Radiation Research 80: 556-562, 1979.

4. Hunt, W. A., Majchrowicz, E., Dalton, T. K., Swartzwelder, H. S., and Wixon, H. N. Alterations in neurotransmitter activity after acute and chronic ethanol treatment: Studies of transmitter interactions. Alcoholism: Clinical and Experimental Research 3: 359-363, 1979.

5. Hunt, W. A., Redos, J. D., Dalton, T. K., and Catravas, G. N. Alterations in brain guanosine-3',5'-cyclic monophosphate levels after acute and chronic treatment with ethanol. Journal of Pharmacology and Experimental Therapeutics 201: 103-109, 1977.

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ED50 AND DOSE-RESPONSE CURVE FOR EARLY TRANSIENT INCAPACITATION FOR A PHYSICALLY DEMANDING TASK

Principal Investigators: C. G. Franz and R. W. Young
Technical Assistance: L. Clark

Extensive dose-response studies have been done to determine the effect of mixed fission-spectrum radiations on the performance of a visual discrimination task by monkeys (Macacca mulatta) seated in primate chairs. Preliminary work with monkeys performing a physical activity task on a nonmotorized treadmill indicated that ionizing radiation may degrade physical activity more severely than it degrades cognitive discrimination (1). The present experiment was designed to test that observation by establishing a dose-response curve and a median effective dose for early transient incapacitation for the physical activity wheel task after whole-body irradiation ($n/\gamma = 3$).

To establish a behavioral baseline against which radiation effects could be measured, techniques described in earlier reports (1,2) were used to train 39 rhesus monkeys to a high degree of stability in performing a physical activity wheel task. After preirradiation performance was carefully established, each subject was exposed to a single pulse of mixed-spectrum whole-body radiation from the AFRRI TRIGA reactor. The field was moderated by 2 inches of lead to produce an incident field with an n/γ ratio of 3. The subjects were tested on a cycle of 10 min work/5 min rest for 6 hours after irradiation. The median effective dose for early transient incapacitation in monkeys performing the physical activity wheel task was 1982 rads (referenced to midline thorax). This is significantly less than the median effective dose of 2771 rads in the same radiation field for the visual discrimination task. The dose-response curve is illustrated in Figure 1.

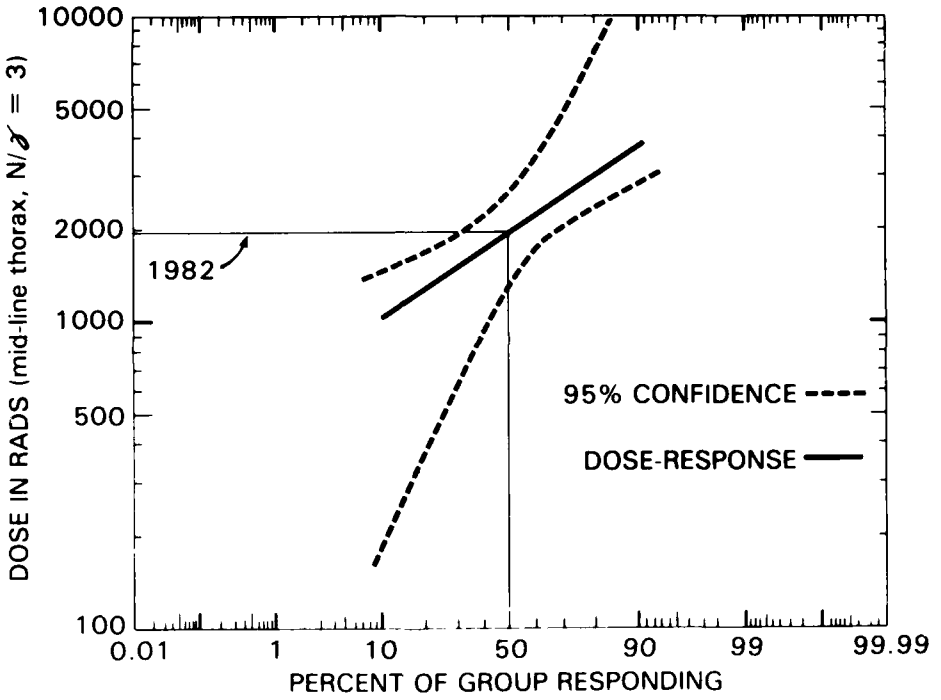

Figure 1. Dose-response curve for early transient incapacitation in the physical activity wheel task

REFERENCES

1. Curran, C. R. and Franz, C. G. Primate physical activity following exposure to a single 2000-rad pulsed dose of mixed gamma-neutron radiation. Scientific Report SR74-2, Armed Forces Radiobiology Research Institute, Bethesda, Maryland, 1974.

2. Franz, C. G., Clark, L., and Cable, J. W. Primate physical activity following exposure to a single 4600-rad pulsed dose of mixed gamma-neutron radiation. Scientific Report SR76-21, Armed Forces Radiobiology Research Institute, Bethesda, Maryland, 1976.

DEVELOPMENT OF A BEHAVIORAL DATA BASE FOR THE AFRRI

Principal Investigators: R. W. Young, C. G. Franz, and W. E. Mitchell

During the past 10 years, the Behavioral Sciences Department has been conducting a research program to investigate the effects of ionizing radiations on performance. The majority of these studies have used the rhesus monkey (Macacca mulatta) as a model for man. The program includes 18 studies made to determine how behavioral performance may be affected by differences in dose, dose rate, quality of radiation, and portion of the subject irradiated. In most of this work the behavioral task has been kept constant while the parameters of radiation exposure have been varied. In the remainder of the work, the task was changed while the radiation parameters were held constant. The data from these studies allow the comparison not only of various radiation exposures on a single behavioral endpoint, but also of the same radiation exposure on different behavioral endpoints. These data are a unique, comprehensive source of information on the direct effects of ionizing radiations on performance. The data can help to provide answers to new operational questions as they arise.

A computerized data base is being established to make this information accessible. It will permit data consolidation, computerized search, and comprehensive analysis. The information to be included represents data on more than 600 subjects and will be searchable on combinations of 37 different selection criteria. Search parameters for the BHS Data Base are listed below.

1	AGE	20	ETI TIMES
2	WEIGHT	21	DOSE MONTH
3	HEIGHT	22	DOSE DAY
4	GIRTH	23	DOSE YEAR
5	SEX	24	DOSE
6	TASK	25	NEUTRON-GAMMA RATIO
7	SPECIES	26	TAR
8	# OF DOSES	27	PROPOSED DOSE
9	REFERENCE POINT	28	TIME
10	# OF EMESES	29	WHOLE OR PART OF BODY
11	PCI HOURS	30	SOURCE
12	PCI MINUTES	31	MODE
13	SURVIVAL DAYS	32	DOSE RATE
14	SURVIVAL HOURS	33	JULIAN DATE
15	SURVIVAL MINUTES	34	# OF EMESES FOR THIS DOSE
16	# OF ETI'S	35	# OF ETI'S FOR THIS DOSE
17	# OF TREATMENTS	36	SPLIT DAYS
18	TYPE OF TREATMENT	37	SPLIT MINUTES
19	EMESES TIMES		

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NEUROBEHAVIORAL ANALYSIS OF DRUG AND RADIATION EFFECTS

Principal Investigator: H. Teitelbaum
Technical Assistance: J. F. Lee and B. A. Dennison

Changes in electroencephalograph amplitude and frequency have been recorded in a number of cortical and subcortical brain regions. After exposure to very high doses of ionizing radiation, variability occurs in response (regarding locus of perturbation) from one animal to another. Although some diencephalic structures are usually associated with early transient incapacitation (medial thalamus, caudate), other structures are rarely affected (hippocampus, parietal cortex). Dose-response studies are being conducted to determine threshold doses at each affected structure.

Autoradiographic measurement of the utilization of brain glucose by ^{14}C-de - glucose incorporation have shown that yohimbine (a drug that produces effec - on performance similar to radiation) acts to inhibit glucose utilization in the l - thalamus. The effect of radiation on glucose uptake of hypothalamus and sub - tia nigra with electrical stimulation of those regions in normal and irrad l subjects is presently being studied.

Perhaps our most promising efforts have been in the production of behavioral incapacitation in the laboratory rat by means of direct microinjections of histamine into the ventricles. So far, we have seen effects similar in time course and magnitude of effect to blood pressure changes and avoidance performance after radiation. Furthermore, there appears to be tolerance to histamine with no cross-tolerance to radiation effects.

REFERENCE

1. Mickley, G. A. and Teitelbaum, H. Persistence of lateral hypothalamic-mediated behaviors after a supralethal dose of ionizing radiation. Aviation, Space, and Environmental Medicine 49: 868-873, 1978.

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BIOCHEMISTRY DEPARTMENT

The Biochemistry Department was established in 1976. Its main research objectives are (a) to elucidate the mechanisms whereby ionizing radiation interferes with the mammalian organism either alone or in combination with other factors such as non-ionizing radiation and chemical agents, and (b) to develop reliable techniques and methodologies for detecting and evaluating radiation-induced changes in biologic systems and extrapolating them to man. Special emphasis is on developing biochemical indicators that can be used to quantify and predict the severity of radiation-induced injury.

The Department is divided into the Physiological Chemistry Division, the Molecular Biology Division, and the Immunological Chemistry Division.

The Physiological Chemistry Division is mainly concerned with biochemical indicators of radiation damage, biologic effects of low-level radiation, and radiation-induced immunosuppression. A number of approaches are used in this research. Investigations continue on radiation-induced changes in serum glycoproteins, protein-bound carbohydrates, and trace metals as significant indicators of radiation damage. Collaborative studies with the National Cancer Institute of the National Institutes of Health concern the immunological effects of radiation and the use of various immunostimulant drugs as potential radioprotectors. This research effort also includes portal (partial) irradiations to determine if there are different degrees of radiation-induced immunosuppression in different lymphoreticular tissues.

Primary aims of the Molecular Biology Division are the elucidation of biochemical mechanisms of damage induced by ionizing radiation alone or in combination with non-ionizing radiation and chemical agents. The effects of radiation and chemical agents such as commercially available organophosphorus compounds on the mammalian central nervous system are also being investigated. Emphasis is given to the radiation-induced damage to cellular membranes, especially lysosomal membrane constituents, because of the importance of lysosomes for the well-being of the cell and therefore of the organism. Research efforts are directed toward purification and elucidation of the mechanism of action of a humoral factor isolated from the blood of lethally irradiated experimental animals; the humoral factor appears to be responsible for the cardiac failure observed after irradiation. The effects of radiation on histamine release and the mechanisms responsible for this release are also being investigated.

Research objectives of the Immunological Chemistry Division include studies on the isolation of hematopoietic stem cells and measurement of their potential as modifiers of radiation damage. By developing an antiserum specific for only the hematopoietic stem cell, it should be possible to specifically "tag" the stem cell with a fluorescent dye. The fluorescent antibody-stem cell complex can then be separated from the remaining hematopoietic cells using the fluorescence-activated cell sorter (FACS-II). The potential of this purified stem cell population as a modifier of radiation damage will then be investigated. A mutant mouse model that is extremely sensitive to radiation is also used in these studies.

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EFFECT OF LOW-LEVEL GAMMA RADIATION ON RIBONUCLEIC ACID POLYMERASE ACTIVITY IN THE DEVELOPING RAT

Principal Investigators: D. E. McClain, J. M. Mitchell, and G. N. Catravas

Biochemical studies by several investigators have revealed that the developmental rise in certain enzyme activity [ribonucleic acid (RNA) content, deoxyribonucleic acid (DNA) content, and protein synthesis] is depressed after prenatal and neonatal irradiation in mammals (1). A generally accepted hypothesis has been that radiation at low-dose levels affects enzyme synthesis quantitatively and/or qualitatively rather than affecting the structure and activity of preexisting enzyme molecules. The belief that DNA degradation is one of the first metabolic events following irradiation suggests that radiation effects in an organism are initiated in the machinery of nucleic acid metabolism. This may involve a radiation-induced cross-linking, a deletion of bases in the DNA, or a DNA-protein interaction involving either chromatin protein or nuclear protoplasmic proteins in such a way that the recognition of certain nucleotide sequences by protein regulators or polymerase enzymes is impaired.

Currently it appears that the most sensitive and informative approach in assessing the quality and quantity of DNA damage is to study the transcription and translation products of that damage. Ribonucleic acid polymerase (RNAp) is of particular interest in studying radiation effects in the prenatal and newborn animal. The process of differentiation appears to be characterized by a carefully scheduled modulation of the genome so that the proper messenger RNA molecules appear at the right time, followed by specific enzymes. The difficult-to-detect in vivo effects of low-level radiation might be more easily seen in this delicately balanced system in the developing animal.

Newborn, male, Sprague-Dawley rats were exposed to 200-rad doses of cobalt-60-gamma radiation. A single dose was given 24 hours after birth. RNAp activity was assayed by sacrificing groups of animals over a period of up to 45 days post-irradiation. Assays were performed on purified intact nuclei isolated from the livers and brains of irradiated and nonirradiated animals. The assay was modified from that of Roeder and Rutter (2).

At this time, baseline activities have been established for the sham-irradiated animals. Brain nuclei from 1-day-old animals show RNAp activity of 0.5 units (ng uridine triphosphate incorporated per mg DNA per minute), which rises quickly to 1.5 units at day 4, before beginning a fairly smooth decline to 0.5 units at day 45. Liver nuclei show a smooth rise in RNAp activity from 2.0 units at day 1 to 3.0 units at day 45. RNAp activities from irradiated animals will ultimately be compared to these control values. Attempts will then be made to determine the nature of the changes (if any) observed in the irradiated animals.

REFERENCES

1. de Vellis, J., Schjeide, O. A., and Clemente, C. D. Protein synthesis and enzymic patterns in the developing brain following head x-irradiation of newborn rats. Journal of Neurochemistry 13: 75-84, 1967.

2. Roeder, R. G. and Rutter, W. J. Multiple acid polymerases in ribonucleic acid synthesis during sea urchin development. Biochemistry 9: 2543-2554, 1970.

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STUDIES ON MODE OF ACTION OF A CIRCULATING MYODYNAMIC AGENT RELEASED BY RADIATION INSULTS

Principal Investigators: J. M. Mitchell, M. Porvaznik, and G. N. Catravas, *AFRRI*
R. N. Hawkins, *Naval Medical Research Institute*

A substance that depresses cardiac contractility has been partially purified by molecular exclusion chromatography. The substances has been named circulating myodynamic agent (CMA). It was isolated from pooled feline plasma at 3 hours after whole-body irradiation of 10,000 rads (165 rads/sec) of high-energy electrons (18-MeV source) and assayed with isolated rat atrial muscle. The atrial muscles were bathed in Krebs-Hanseleit buffer, pH 7.2, aerated with 95% dioxide and 5% carbon dioxide at 30°C. Atrial muscle preparations having a resting tension of 1.25 g were stimulated 1 volt above threshold at 2.5 hertz and a pulse duration of 200 msec. A 1% depression in contractile force was defined as 1 unit (U) of depressant activity/ml of bathing solution. A CMA concentration of 40 U/ml was used for each experiment. Approximately 1 min after addition of CMA to the muscle preparations, a 40% reduction in contractile force was observed. A partial recovery in contractile force followed this initial depression. A second decrease in contractile force began approximately 12 min after the addition of CMA, and was completed by the end of the 20-min assay period.

Atrial muscle preparations were fixed for freeze fracture in 2.5% glutaraldehyde buffered in Krebs-Henseleit, pH 7.2, aerated with 95% O_2/5% CO_2 at 30°C. Complete loss of stimulated contraction occurred at 1 min after addition of the fixative bath. The mean center-to-center particle spacing of control gap junctions was 10.1 $\pm$ 0.8 (SD) nm. The mean center-to-center particle spacing at 1 min after addition of the cardiac depressant was 9.62 $\pm$ 1.15 nm, and after 6 min was 9.62 $\pm$ 0.67 nm. Significant differences in distribution of particle spacings within the gap junctions at 1 min after addition of CMA ($p = 0.001$) and after 6 min ($p = 0.0005$) have led us to suggest that individual sarcomeres may have become partially uncooked, causing a depression in cardiac contractility.

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CALCIUM CHANGES DURING HISTAMINE RELEASE DERIVED FROM ADENOSINE TRIPHOSPHATE IN MAST CELLS

Principal Investigator: M. A. Donlon, *AFRRI*
Collaborators: G. N. Catravas, *AFRRI*
M. A. Kaliner, *National Institute of Allergy and Infectious Diseases, National Institutes of Health*
Technical Assistance: C. E. Bland, *NIAID, NIH*

Although several reports have demonstrated the release of histamine from mast cells after radiation, no explanations have been advanced regarding the mechanism of this response. We have investigated aspects of histamine release in relation to calcium transport and stimulation of secretion using adenosine triphosphate (ATP) in normal isolated rat peritoneal mast cells. We have previously described two cell-associated calcium (CAC) compartments that are altered by stimulation of secretion and histamine release. The total CAC pools have been identified by a brief ethanedioxy-bis-(ethylamine)-tetraacetic acid (EGTA) treatment of cell suspensions after exposure to calcium-45 in the presence and absence of various concentrations of ATP. The results are shown in Figure 1.

Purified (>90%) rat mast cells were incubated at 37°C in buffer solutions containing calcium-45 and various ATP concentrations. Half of the cell suspension was exposed to EGTA for 2 min, and both samples were rapidly centrifuged through silicone oil. The supernatant was assayed for histamine release, and the pellet was analyzed for calcium-45 content. Increases in the internal CAC pool are associated with histamine release, with a maximum histamine release seen at 1 mM ATP. High concentrations of ATP (10 mM) inhibit both histamine release and any increases in calcium pools.

These data further support the hypothesis that increases in internal calcium pools will regulate histamine release and will show increases in external calcium-binding sites associated with the process of secretion.

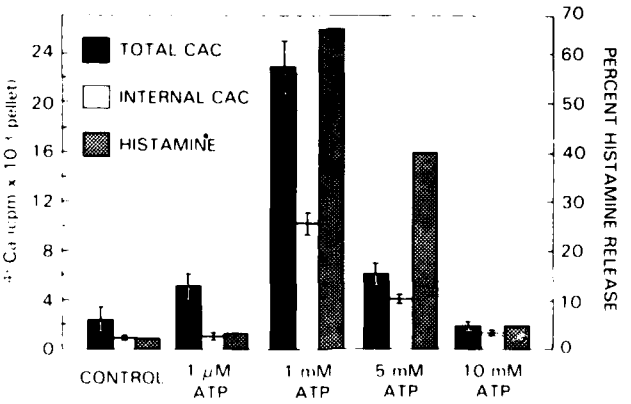

Figure 1. Effect of various concentrations of ATP on cell-associated calcium compartments (CAC) and histamine release in isolated rat peritoneal mast cells

CALCIUM TRANSPORT IN RAT PERITONEAL MAST CELLS

Principal Investigator: M. A. Donlon, *AFRRI*
Collaborators: G. N. Catravas, *AFRRI*
M. A. Kaliner, *National Institute of Allergy and Infectious Diseases, National Institutes of Health*

It has been a consistent observation that radiation induces histamine release in humans as well as in a variety of experimental animals. Mast cells (the primary site for histamine stores) are widely dispersed throughout the body but occur most abundantly in the area of small blood vessels, nerves, and glandular ducts.

High doses of radiation produce a histaminic shock syndrome that has been related to early transient incapacitation. The mechanism that elicits cell degranulation of mast cells in response to radiation injury remains an enigma. All known substances that cause the release of histamine act at the external cell membrane to initiate a series of biochemical events, which ultimately results in an increase in intracellular calcium levels.

This study focuses on defining the calcium association with purified rat peritoneal mast cells (RPMC) in relation to histamine release stimulated by compound 48/80. The results are shown in Figure 1.

Purified RPMC's were incubated at 37°C for 5 min in medium containing calcium-45 and various concentrations of 48/80. Cell suspensions were centrifuged through silicone oil before and after treatment with ethanedioxy-bis-(ethylamine)-tetraacetic acid (EGTA) to remove external calcium-45. The pellet was analyzed for radioactivity and the supernatant was assayed for histamine release. The total cell-associated calcium compartment increases dramatically on the stimulation of secretion by compound 48/80. A correlation between internal cell calcium and histamine release is evident.

The data support the hypothesis that the changes in internal cell calcium concentration are related to the extent of histamine released from mast cells.

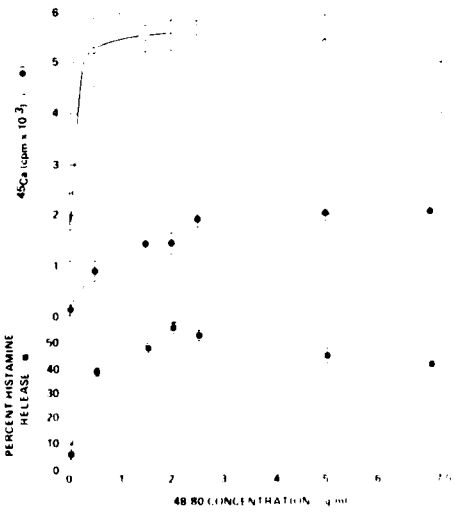

Figure 1. Relationship between histamine release and cell calcium compartments. Cell-associated radioactivity in calcium internal pools (●) and total calcium pools ( ): histamine release (■) in isolated rat peritoneal mast cells stimulated with compound 48/80. Each point represents mean of triplicate samples ± SE.

EFFECTS OF RADIATION ON LEVELS OF CYCLIC AMP, CYCLIC GMP, AND AMINO ACID IN CEREBROSPINAL FLUID OF THE PRIMATE

Principal Investigators: G. N. Catravas, S. J. Wright, Jr., P. J. Trocha, and J. K. Takenaga

Previous studies have indicated that exposure of animals to ionizing radiation affects the adenyl cyclase system in different tissues. Decreases in adenyl cyclase and phosphodiesterase activities have been observed in the liver of newborn rats exposed to low levels of radiation (1). On the other hand, no changes in cyclic adenosine monophosphate (AMP) levels were observed in irradiated thymocytes with gamma radiation, whereas heating at 43°C was found to cause a massive rise in its levels within the cell (2). Little information is available on the effects of radiation on cyclic nucleotide metabolism in the mammalian cerebrospinal fluid. Therefore, the purpose of this study was to determine if and to what extent cyclic AMP and cyclic guanosine monophosphate (GMP) levels in cerebrospinal fluid are affected by the exposure of animals to ionizing radiation.

Silastic Pudenz catheters were chronically implanted in the fourth ventricle of cynomolgus monkeys and were connected to compressible polyethylene Ommaya reservoirs placed subcutaneously over the occiput. Cerebrospinal fluid could be asceptically aspirated from the reservoir in the awake animal. Before irradiation, duplicate baseline samples were taken 24 hours apart after repeated reservoir pumping to ensure good mixing with the cerebrospinal fluid in the ventricle. Heads of the animals were then exposed to 900 rads of 6.5-MeV bremsstrahlung from the AFRRI linear accelerator. The average dose rate was 70 rads/min. Cerebrospinal fluid samples were taken at 1, 24, 48, and 72 hours after irradiation. Sample collection time was at 1300 hours.

Significant increases in the levels of both cyclic AMP and cyclic GMP were observed after irradiation. No appreciable changes were found in the amino acid composition of the cerebrospinal fluid.

REFERENCES

1. Slozhenikina, L. V., Michailets, L. P., Fialkovskaia, L. A., and Kuzin, A. M. Hepatic adenosine-3',5'-monophosphate metabolism in newborn rats irradiated during the period of organogenesis. Bulletin of Experimental Biology and Medicine 86: 91-93, 1978.

2. Lin, P. S., Kwock, L., Hefter, K., and Wallach, D. F. H. Modification of rat thymocyte membrane properties by hyperthermia and ionizing radiation. International Journal of Radiation Biology 33: 371-382, 1978.

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EFFECTS OF MORPHINE ON LEVELS OF CYCLIC GMP IN CEREBROSPINAL FLUID AND CEREBELLUM OF THE MONKEY

Principal Investigators: G. N. Catravas, S. J. Wright, Jr., and J. B. Katz

Biochemical indications that cyclic guanosine monophosphate (GMP) may play an important role in cerebellar function are its high concentration in the cerebellum, a high-affinity cyclic GMP-binding protein in rat cerebellum, and a distinct cyclic GMP-dependent protein kinase in bovine cerebellum. Morphine, pentobarbital (1), and ethanol (2) administered systemically have been shown to depress the levels of cyclic GMP in rat cerebellum.

Through in vitro enzymatic studies, we attempted to explain the reduction of levels of cerebellar cyclic GMP after the administration of morphine. Although exhaustive study of cerebellar guanylate cyclase and phosphodiesterase was not undertaken, a variety of experiments failed to reveal depression of guanylate cyclase or stimulation of phosphodiesterase activities in cerebella removed from acutely morphine-intoxicated rats. Such changes might have explained the observed diminution of levels of cyclic GMP. However, levels of cerebellar cyclic GMP could perhaps change as a consequence of the exit of cyclic GMP from cerebellar tissue into surrounding cerebrospinal fluid. Therefore, we decided to determine if levels of cyclic GMP in cerebrospinal fluid would rise upon morphine administration.

Silastic Pudenz catheters were chronically implanted in the fourth ventricle of monkeys and were connected to compressible polyethylene Ommaya reservoirs placed subcutaneously over the occiput for aspiration of cerebrospinal fluid. Administration of morphine (20 mg/kg intramuscularly) to the awake animal significantly elevated the levels of cyclic GMP in the cerebrospinal fluid. After hemicraniectomy, biopsies of cerebral and cerebellar cortex were taken from monkeys under anesthesia (20 mg/kg morphine sulfate given intramuscularly). Only the cerebellar cyclic GMP levels were found to change significantly, showing a more than 30% decrease compared to anesthetized controls. Naloxene (0.3 mg/kg intramuscularly) blocked the changes observed in levels of cyclic GMP in both the cerebrospinal fluid and the cerebellum. Although the controlling factors for levels of cyclic GMP in brain and cerebrospinal fluid are not well understood, our results indicate that, under some conditions, a reciprocal relationship may exist between cyclic GMP levels in certain brain regions and in cerebrospinal fluid.

REFERENCES

1. Katz, J. B. and Catravas, G. N. Cerebellar cGMP levels reduced by morphine and pentobarbital on a dose- and time-dependent basis. Biochemical Pharmacology 25: 2543-2546, 1976.

2. Redos, J. D., Catravas, G. N., and Hunt, W. A. Ethanol-induced depletion of cerebellar guanosine 3',5'-cyclic monophosphate. Science 193: 58-59, 1976.

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EFFECTS OF ELECTROMAGNETIC RADIATION (BREMSSTRAHLUNG) AND DEXAMETHASONE ON LEVELS OF PROTEIN - BOUND CARBOHYDRATES IN SERUM OF THE PRIMATE

Principal Investigators: G. N. Catravas, A. N. Martins, R. E. Severance, and T. F. Doyle

Previous investigations have shown that the levels of certain protein-bound carbohydrates in the sera of mice and dogs increase after whole-body irradiation with 14-MeV neutrons or mixed neutron-gamma radiation (1). On the other hand, electrophoretic studies revealed that the levels of certain serum proteins, some of which contain carbohydrates in their molecule, decrease after exposure to lethal doses of electromagnetic radiation (2). We had the opportunity to further study this phenomenon as part of an investigation (previously reported from this laboratory) that explored the possibility of interaction between (a) administration of high doses of glucocorticoid dexamethasone and (b) irradiation of the brain. The purpose of the present study is to determine if irradiation of only the cranial vault will result in any changes in the levels of serum protein carbohydrate components, and what effect, if any, the administration of dexamethasone will have on those levels.

Male, rhesus monkeys were exposed to 1800 rads of 6.5-MeV bremsstrahlung from the AFRRI linear accelerator, to the area of only the brain. The average dose rate was 213 rads per minute. Portal (partial) irradiation of the cranial vault was effected through an aperture in a 3-inch-thick lead shield of the approximate size and shape of the brain. One half of the total number of animals were injected with 4 mg dexamethasone (intramuscularly) twice daily for 11 days, beginning 1 day before irradiation. The dose was then gradually reduced to 0.4 mg at the end of the 21st day, when administration of the glucocorticoid was discontinued. The remaining animals were injected with equal volumes of physiologic saline, under identical conditions.

Gradual decrease in the levels of protein-bound sialic acid in the serum of the saline-treated control animals was observed after irradiation (Figure 1). Compared to preirradiation levels, the reduction reached statistically significant values ($t < 0.01$) during the 11th week postirradiation. No other significant changes in the content of sialic acid were observed. As shown in the same Figure, a similar decrease in sialic content occurred in the group of animals that had received dexamethasone in addition to radiation. However, no statistically significant differences in sialic content were found between the group treated with dexamethasone and the saline-treated controls.

A drastic decrease in the levels of protein-bound neutral hexoses was observed in the serum of the saline-treated control animals at 6 days after irradiation ($t < 0.005$), followed by a slight increase during the second week postirradiation (Figure 2). The levels of neutral hexoses then decreased gradually, until they reached their lowest values on the 11th week postirradiation. As in the case of sialic acid, there was another slight increase in their levels during the 15th week; by the end of the experiment, the levels returned to those of the 11th week postirradiation. A similar pattern of radiation-induced changes was observed in the animals treated with dexamethasone. However, as in the case of sialic acid, no statistically significant differences were found in levels of neutral hexose between the group treated with dexamethasone and the controls treated with saline.

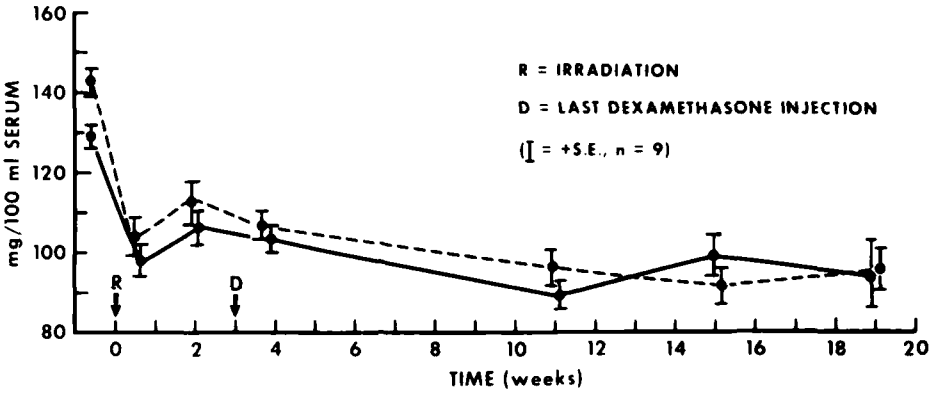

Figure 1. Effects of bremsstrahlung irradiation of brain on protein-bound N-acetyl neuraminic acid levels in serum. (●——●), saline-treated; (●---●), dexamethasone-treated.

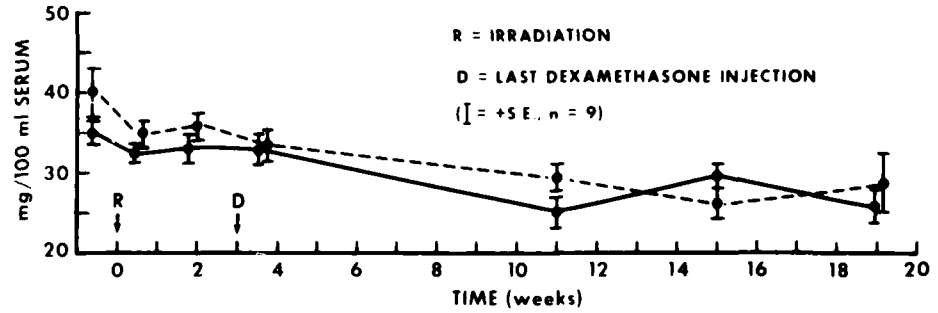

Figure 2. Effects of bremsstrahlung irradiation of brain on protein-bound neutral hexoses levels in serum. (●——●), saline-treated; (●---●), dexamethasone-treated.

REFERENCES

1. Evans, A. S., Brown, J. A., and Strike, T. A. Effects of ionizing radiation on total protein-bound neutral hexoses in the plasma of mice. Radiation Research 36: 128-137, 1968.

2. Sassen, A., Kennes, F., and Maisin, J. R. Roentgen irradiation effects on mouse proteins. Acta Radiologica; Therapy, Physics, Biology 4: 97-112, 1966.

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EFFECT OF IRRADIATION WITH HIGH-POWER-DENSITY MICROWAVES ON SOLUBLE PROTEINS OF THE RABBIT LENS

Principal Investigators: G. M. Oosta and N. S. Mathewson

Cataracts can be induced in the ocular lens by a variety of agents, including exposure to microwaves of sufficient duration and power density (1,2). However, the mechanism of formation of the microwave cataract has remained obscure.

We report the use of quantitative pore-gradient electrophoresis to measure the distribution of soluble rabbit-lens protein in normal lenses and in lenses that had received many exposures to high-power-density microwaves. The results suggest that quantitative pore-gradient electrophoresis is useful for measuring small changes in the distribution of soluble lens protein and that it may be a suitable technique for probing the mechanism behind formation of cataracts.

New Zealand rabbits were irradiated on the left side of the head by microwaves (2.45 GHz) at 300 mw/cm^2 for 20 min on each of 2 consecutive days. Observed by biomicroscopy, the lens changes in irradiated animals ranged from no changes to small posterior subcapsular opacities. When pore-gradient electrophoresis was used, a marked difference was observed in the distributions of soluble lens proteins in the lens cortex and nucleus. Comparison of irradiated and control lenses revealed an apparent shift toward components of higher molecular weight in the cortex samples of lenses irradiated with microwaves.

REFERENCES

1. Carpenter, R. L. and Van Ummerson, C. A. The action of microwave radiation on the eye. Journal of Microwave Power 3: 3, 1968.

2. Kramer, P., Guy, A., Emery, A., and Harris, C. Acute microwave radiation and cataract formation in rabbits and monkeys. Journal of Microwave Power 11: 136, 1976.

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INFLUENCE OF 45-Hz VERTICAL ELECTRIC FIELDS ON GROWTH, FOOD AND WATER CONSUMPTION, AND BLOOD CONSTITUENTS OF RATS

Principal Investigators: N. S. Mathewson, G. M. Oosta, S. A. Oliva, S. G. Levin, and S. S. Diamond

Extremely low frequency (ELF) radiation generally denotes electromagnetic radiation having frequencies from a few hertz (sometimes including direct current) to several hundred hertz. The natural levels of ELF radiation have been found to be generally less than 0.01 V/m. In contrast, man-made ELF electric fields in the home or office, which arise principally from operating electrical appliances, can

reach 250 V/m. Under power transmission lines, ELF electric fields can be on the order of 10^4 V/m.

Recently Marino et al. reported that reduction of growth will result when rats (1) or mice (2) are exposed to 60-Hz electric field strengths near 10^4 V/m. In contrast, Knickerbocker et al. (3) exposed mice to 60-Hz fields (157,000 V/m) and found no growth alteration. We report an experiment in which rats were exposed to 45-Hz vertical electric fields at field strengths of up to 100 V/m. Our results suggest that there are no biologically important differences in growth rate, food and water consumption, selected blood metabolite concentrations, constituents of a complete blood count, or histopathology of selected tissue samples.

Young, male rats, weighing approximately 180 g, were exposed to 45-Hz vertical electric fields in nonmetallic cages during four experiments. In each of three experiments, six groups of 16 animals were exposed to field strengths of 0, 2, 10, 20, 50, and 100 V/m for 28 days. In the fourth 28-day experiment, 48 animals were exposed to 20 V/m, and 48 were controls. Statistical analysis reveals no consistently reproducible differences ($p < 0.05$) between controls and irradiated animals in growth, food consumption, or water consumption. Further, no consistent, reproducible differences ($p < 0.05$) are found for serum or plasma concentrations of total protein, globulin, glucose, cholesterol, triglycerides, total lipids, or constituents of a complete blood count. In addition, necropsy and histopathological examination of tissue from 16 organs failed to reveal any changes that could be attributed to ELF electric fields.

REFERENCES

1. Marino, A. A., Berger, T. J., Austin, B. P., and Becker, R. O. Evaluation of electrochemical information transfer system. I. Effect of electric fields on living organisms. Journal of Electrochemical Society 123: 1199-1200, 1976.

2. Marino, A. A., Becker, R. O., and Ullrich, B. The effects of continuous exposure to low frequency electric fields on three generations of mice: A pilot study. Experientia 32: 565-566, 1976.

3. Knickerbocker, G. G., Kouwenhoven, W. B., and Barns, H. C. Exposure of mice to a strong AC electric field--An experimental study. IEEE Transactions on Power Apparatus and Systems PAS-86: 498-505, 1967.

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PROPERTIES OF SERUM GLYCOPROTEINS AFFECTED BY RADIATION

Principal Investigators: J. F. Weiss and C. E. Elhardt, *AFRRI*
Collaborator: P. B. Chretien, *National Institutes of Health*
Technical Assistance: J. C. Jeng and C. M. Morris

Elevations in serum glycoproteins and protein-bound carbohydrates are significant consequences of radiation damage, trauma, and other disease states. There are also serum glycoproteins, such as α_2HS-glycoprotein, that are depressed due to trauma. Studies on changes in these serum glycoproteins are important in relation to (a) their usefulness as potential biochemical markers of radiation damage and accompanying injuries and (b) the functional significance of these changes.

Recent emphasis in these studies has been on elucidating the hypothesized immunomodulatory effects of certain serum proteins, especially α_2HS-glycoprotein, with the practical aim of diagnosis and treatment of immunosuppression such as that accompanying exposure to radiation. A number of studies were described (1) that indicated the immunological significance of α_2HS-glycoprotein. Data from several studies related serum levels of α_2HS-glycoprotein to assays of cell-mediated immunity. Of the normal serum proteins studied, α_2HS-glycoprotein correlated most strongly with both in vivo and in vitro assays of cell-mediated immunity. It correlated directly with skin test reactivity (delayed cutaneous hypersensitivity to dinitrochlorobenzene), lymphocyte reactivity to phytohemagglutinin, and levels of T-lymphocytes ($p < 0.001$). Levels of T-cells and α_2HS-glycoprotein were simultaneously depressed during immunosuppressive radiotherapy or chemotherapy.

Techniques for the isolation of α_2HS-glycoprotein from blood were compared (Table 1), and preliminary studies were done on the chemical properties (isoelectric focusing) and biologic properties of the isolated protein. Studies with fluoresceinated antisera to α_2HS-glycoprotein indicated specificity of binding to α_2HS-glycoprotein to subpopulations of white blood cells. Studies by others indicating the stimulation of macrophage phagocytosis by α_2HS-glycoprotein are supported by our preliminary evidence of chemotactic properties and of stimulation of macrophage activity by α_2HS-glycoprotein.

Table 1. Procedures for Isolating α_2HS-Glycoprotein

Burgi & Schmid (1961)	van Oss et al. (1974)	
Plasma Fractionazation By Method 6 of Cohn, Supernatant IV-4 ethanol 40 %, pH 4.8, -5° Supernatant V lyophilize, pH 5.8, ethanol 20 %, barium acetate 0.02 M Ba α_2 GP dissolve in EDTA 0.1 M, pH 7, dialyze lyophilize Ba α_2 or α_2HS GP yield 1.8 %	Diluted Plasma, pH 5 4°, dialyze vs. Na acetate 0.03 M, pH 5 Supernatant DEAE cellulose adsorption, Na acetate 0.3 M pH 5 Eluate curtain electrophoresis, pH 8.6, 2 X Extract sephadex G-200 Post Third Peak $(NH_4)_2SO_4$ 2.4 M α_2HS GP yield 0.5 %	Plasma affinity chromatography Bound Protein elution Eluate preparative isoelectric focusing α_2HS GP yield 12%

REFERENCE

1. Weiss, J. F., Elhardt, C. E., Baskies, A. M., Wolf, G. T., and Chretien, P. B. Serum α_2HS-glycoprotein: A biochemical correlate of cellular immunity. In: Abstracts of Papers. American Chemical Society, Washington, D.C., 1979, Abstract no. BIOL-169.

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RADIATION AND SERUM METALS

Principal Investigators: W. P. Bradley and J. F. Weiss, *AFRRI*
Collaborator: P. O. Alderson, *Johns Hopkins Hospital*
Technical Assistance: C. L. Harding

Serum metal concentrations have been observed to change after irradiation. Radiation effects on concentrations of serum metal were studied at AFRRI, with early emphasis on the radionuclide gallium-67. The biodistribution of gallium-67 seems to be strongly influenced by the presence of iron-binding proteins such as transferrin and lactoferrin.

Our initial studies showed that whole-body irradiation increases the serum iron levels and reduces the unsaturated iron-binding capacity, resulting in decreased tissue uptake of gallium-67 and increased nuclide excretion in the urine. We then studied (1) the effect of iron deficiency on distribution of gallium-67 in male rats maintained on a low-iron diet (3 mg/day). The liver and spleen showed markedly increased uptake of gallium in iron-deficient animals. Femoral bone and marrow uptakes were not altered, but the mean bone-to-blood ratio was higher in the iron-deficient animals due to low blood levels. Urinary excretion of gallium-67 was significantly decreased. Anemic animals given dietary iron showed normal liver-spleen uptake of gallium and increased urinary excretion of gallium-67.

These studies show the importance of iron status on the biodistribution of gallium-67, which can be used to assess various disease states. Knowledge of the interrelationship of serum iron and gallium is necessary in evaluating the usefulness of methods for determining radiation damage when using these metals. Currently, the effects of radiation on the urinary excretion of physiological metals is being investigated.

REFERENCE

1. Bradley, W. P., Alderson, P. O., and Weiss, J. F. Effect of iron deficiency on the biodistribution and tumor uptake of Ga-67 citrate in animals. Journal of Nuclear Medicine 20: 243-247, 1979.

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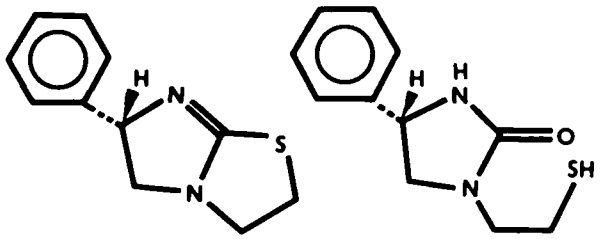
RADIATION CHEMISTRY OF POTENTIAL RADIOPROTECTANTS

Principal Investigators: C. R. Dobbs, C. E. Elhardt, and L. May
Technical Assistance: K. M. Hartley

When a compound is found to be a radioprotectant, either naturally occurring or a drug, the questions arise as to (a) whether the molecular species itself furnishes protection to the organism against ionizing radiation, and (b) whether the drug is first converted by the initial radiation to the radioprotectant species or whether the organism converts the compound to a metabolite that acts as the radioprotectant.

The effect of radiation on levamisole was first studied (1). Levamisole, (S)-(-)-2,3,5,6-tetrahydro-6-phenyl-imidazo-(2, 1-b) thiazole, an immunomodulating drug and veterinary antinelminthic, is converted by tissues to a sulfhydryl derivative (see Figure 1). The drug has been shown to inhibit lipid peroxidation and can be considered a radioprotectant. It was of interest to establish if the sulfhydryl derivative is also a product of the direct action of the ionizing radiation on levamisole. Therefore, we examined the effect of gamma radiation on aqueous solutions of the drug.



LEVAMISOLE O.M.P.I.

A B

Figure 1. Structural formulas of levamisole (A) and its metabolite DL-2-OXO-3-(2-mercapto-ethyl)-5-phenylimidazolidine (OMPI) (B)

Thin layer chromatography and chromagraphic analyses of the radiation products revealed two major products. These were analyzed with spectrophotometry and with gas chromatography-mass spectrometry. Each of the individual major products contained several substances, none of which had the properties of the sulfhydryl derivative. Further purification of the radiation products was made using silicic acid chromatography. The major products were analyzed from data obtained from mass spectra, optical and infrared spectra, and nuclear magnetic resonance spectra.

REFERENCE

1. Dobbs, C. R., Elhardt, C. E., and May, L. Radiation chemistry of levamisole. Applied Spectroscopy 33: 433, 1979.

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MODULATION OF IMMUNOSUPPRESSION RESULTING FROM IRRADIATION

Principal Investigators: J. F. Weiss and K. F. McCarthy, *AFRRI*
Collaborators: M. A. Chirigos and W. A. Stylos, *National Institutes of Health*
Technical Assistance: W. W. Wolfe

Information is needed about (a) the effects of radiation on the various organs and cells involved in immune defense and (b) their interrelationships. This information is important in understanding radiation injury related to the effects of nuclear weapons, including collateral damage and therapeutic doses of radiation. In this work unit we are also investigating a new area of radioprotection: the use of modifiers of biologic response to protect against whole-body or portal (partial-body) irradiation.

The lymphocyte is one of the most radiosensitive cells in the mammalian body. It has been established that subpopulations of lymphocytes differ in their reactivity toward whole-body irradiation. In the current studies (1,2), the effect of whole-body irradiation (X rays, 40 rads/min) on T-cell and B-cell populations of tumor-bearing mice were studied, and the effect of the immunomodulator maleic anhydride-vinyl ether (MVE) or pyran was determined. The splenic T- and B-lymphocyte populations of BALB/c mice were determined in animals bearing Madison lung carcinoma 109. Concurrently, some groups of tumored mice were exposed to 500 rads of whole-body irradiation and were treated with one dose of MVE. By direct immunofluorescence it was found that the percentage of splenic T-lymphocytes was significantly depressed in the tumored-irradiated mice. Mitogenic studies revealed that the T-lymphocytes were more depressed in the tumored-irradiated mice than in the corresponding nonirradiated tumored mice. MVE was relatively effective in reconstituting the T-cell compartment of these splenic T-lymphocytes (Figure 1). The B-cell compartment of the splenic lymphocytes of the tumored-irradiated mice was found to be extremely radiosensitive. Using a specific anti-B serum, no B-lymphocytes were detected during the testing. Blastogenic studies using lipopolysaccharide as the mitogenic probe revealed that the incorporation of ^{3}H-thymidine by tumored-irradiated mice was just slightly higher than background values. MVE proved to be relatively ineffective in reconstituting the splenic B-cells of irradiated mice.

For the purpose of portal (partial) irradiation, an array was designed that allowed 10 immobilized mice at a time to be irradiated through a circle (diameter 2 cm) cut in a lead shield. Using this array, the effect of irradiation of the lungs on the survival of normal or tumor-bearing mice was first investigated (3). Electrons of

18 MeV were delivered from a linear accelerator at an average dose rate of 200 rads/min. Normal mice survived single total doses of 4000 rads. Irradiation of mice (800-1800 rads) bearing lung tumors (M109) resulted in a consistent 45%-70% increase in survival time compared to the nonirradiated animals. The time of irradiation after tumor inoculation did not markedly alter the percentage increase in survival time.

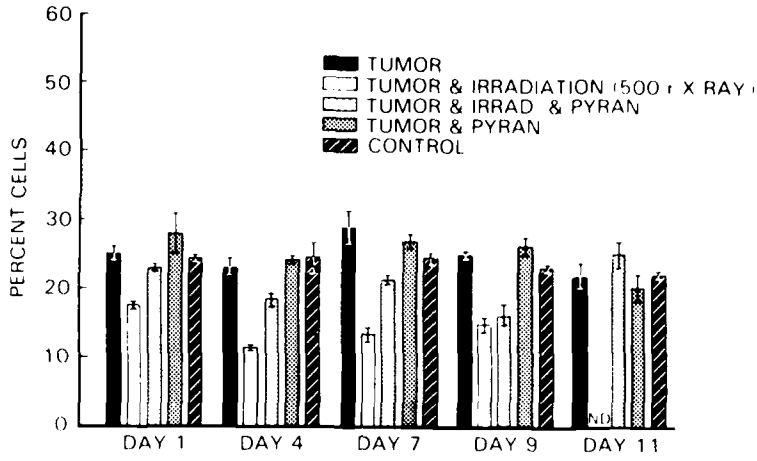

Figure 1. Effect of MVE (pyran) on percentage of positive fluorescence of splenic T lymphocytes in tumored-irradiated mice

REFERENCES

1. Stylos, W. A., Chirigos, M. A., Lengel, C. R., and Weiss, J. F. T and B lymphocytes of irradiated, tumor-bearing mice treated with an immunostimulator, pyran. Cellular Immunology 40: 437-444, 1978.

2. Stylos, W. A., Chirigos, M. A., Lengel, C. R., and Weiss, J. F. Effect of maleic anhydride-vinyl ether on the lymphocyte subpopulations of tumor-bearing irradiated mice. Cancer Treatment Reports 62: 1831-1836, 1978.

3. Weiss, J. F. and Chirigos, M. A. Response of murine M109 alveolar carcinoma to portal irradiation and/or chemotherapy. In: Abstracts of Sixth International Congress of Radiation Research. Japanese Association of Radiation Research, Tokyo, 1979, p. 248.

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ANTIOXIDANT AND RADIOPROTECTIVE PROPERTIES OF LEVAMISOLE

Principal Investigators: K. S. Kumar (NRC Fellow), J. F. Weiss, and C. R. Dobbs, *AFRRI*
Collaborator: M. A. Chirigos, *National Institutes of Health*
Technical Assistance: K. M. Hartley and W. W. Wolfe

During a survey of the radioprotective properties of drugs that affect the immune response, it was observed that the immunomodulator levamisole prolonged the survival of irradiated mice of various strains. A series of studies was done to elucidate the mechanism of the radioprotective properties of levamisole (1-3).

Levamisole was shown to be an antioxidant to rat liver microsomes in vitro (Table 1). At 1.0 and 2.0 mM, levamisole significantly inhibited the lipid peroxidation of rat liver microsomes initiated by reduced nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine diphosphate-iron complex (ADP-Fe) by 48% and 69%, respectively. With ascorbate and ADP-Fe as the initiators of lipid peroxidation, the respective values at these concentrations were 56% and 81%. When microsomes were heat-inactivated, levamisole inhibited the lipid peroxidation (which is nonenzymatic) initiated by ascorbate and ADP-Fe. Lipid peroxidation was not initiated by the enzyme-dependent system NADPH and ADP-Fe. Oxygen consumption with 1.0 and 2.0 mM levamisole was decreased to 82% and 68% of the control with either NADPH or ascorbate and ADP-Fe. Levamisole also inhibited lipid peroxidation induced by X irradiation and ADP-Fe (25% and 55% inhibition with 1.0 and 2.0 mM levamisole, respectively) (Table 1).

The mechanism of the antioxidant effect is probably related to the nonenzymatic formation of a sulfhydryl intermediate. The relation between the antioxidant and the immunological properties of the drug requires further investigation.

Table 1. Levamisole Inhibition of Microsomal Lipid Peroxidation

	% INHIBITION OF TBA REACTIVE MATERIAL		
MICROSOMES + ADP Fe^{+++}	NADPH	ASCORBATE	2500 RADS X RAY
+ 1.0 mM LEVAMISOLE	48 · 11 ($p < 0.01$)	56 · 16 ($p < 0.05$)	25 · 7 (NS)
+2.0 mM LEVAMISOLE	69 · 10 ($p < 0.001$)	81 · 6 ($p < 0.001$)	55 · 4 ($p < 0.01$)

MICROSOMAL LIPID PEROXIDATION MEASURED BY THE FORMATION OF TBA REACTIVE MATERIAL WAS INHIBITED IN INCUBATION MIXTURES CONTAINING ADP Fe AND EITHER NADPH, ASCORBATE OR INDUCED BY IONIZING RADIATION IN THE PRESENCE OF THE PROMOTER, ADP Fe.

REFERENCES

1. Kumar, K. S., Chirigos, M. A., and Weiss, J. F. Radioprotective and antioxidant effects of levamisole on rat liver microsomes. In: Abstracts of Sixth International Congress of Radiation Research. Japanese Association of Radiation Research, Tokyo, 1979, p. 258.

2. Kumar, K. S., Chirigos, M. A., and Weiss, J. F. Protection of rat liver microsomes from NADPH-, ascorbate-, and X-irradiation-induced lipid peroxidation by levamisole. International Journal of Immunopharmacology 1: 85-91, 1979.

3. Kumar, K. S., Dobbs, C. R., Weiss, J. F., and Chirigos, M. A. Inhibition of microsomal lipid peroxidation in vitro by levamisole and the possible mechanism of action. Federation Proceedings 38: 848, 1979.

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LIPID PEROXIDATION INDUCED BY RADIATION

Principal Investigators: C. R. Dobbs, J. F. Weiss, G. N. Catravas, K. S. Kumar (NRC Fellow), and C. F. Elhardt

Technical Assistance: K. M. Hartley

Membrane lipids can be damaged by free radical species produced during radiation. Free radical species (hydroxyl, hydroperoxy, superoxide) attack cells, particularly at the unsaturated sites of cell membrane lipids, and lipid peroxides are produced. The extent of lipid peroxidation during radiation can be studied by measuring the end products of the reactions. We have investigated a unique method of estimating lipid peroxidation after radiation exposure by analyzing volatile hydrocarbons (1).

We first investigated the production of pentane in a microsomal system in which lipid peroxidation was enzymatically induced. When microsomal preparations were incubated at 37°C for 1 hour with or without adenosine diphosphate-iron complex (ADP-Fe), very little pentane was produced. With ADP-Fe and reduced nicotinamide adenine dinucleotide phosphate (enzymatic system), 540 pmoles pentane/mg microsomal protein was produced. Pentane production was almost totally inhibited by the antioxidant butylated hydroxytoluene (BHT), and was inhibited by about 50% (see Figure 1) by the immunoadjuvant levamisole. When microsomes were irradiated with a gamma source in the presence of optimized concentrations of ADP and Fe^{+++}, a large initial increase in production of pentane occurred within the first 100 min at higher gamma-flux (2000 Gy); it increased only gradually thereafter. At lower doses, production of pentane assumed a more linear aspect; gradual increases were noted for at least 300 min after irradiation. Increases in the rate and the amount of production of n-pentane in response to gamma irradiation were mediated or offset by the presence of known antioxidants. The microheterogeneity of microsomal proteins shown by isoelectric

focusing was also affected by radiation. Preliminary studies indicated the suppression of this effect by BHT, suggesting that end products of lipid peroxidation may react with microsomal protein.

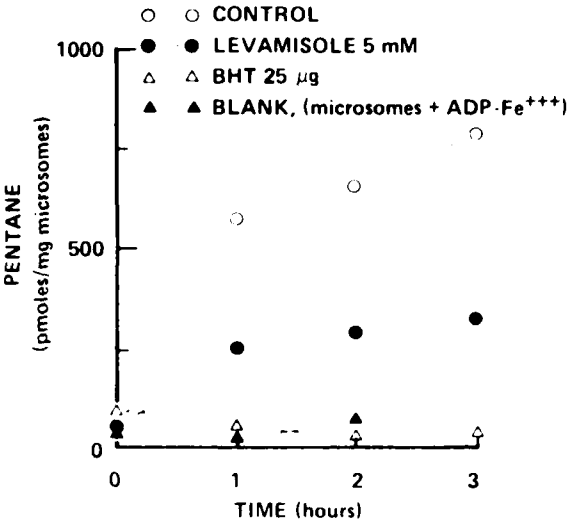

Figure 1. Effect of levamisole and butylated hydroxytoluene (BHT) on production of pentane. Pentane measured in headspace of incubation flasks containing microsomes, reduced nicotinamide adenine dinucleotide phosphate (NADPH), and adenosine diphosphate-iron complex ($ADP\text{-}Fe^{+++}$)

Studies were also conducted on enzymes involved in the scavenging of peroxides and free radicals. Of special interest is the possible role of superoxide dismutase in preventing detrimental effects due to generation of the superoxide radical and the potential radioprotective properties of this enzyme. In preliminary studies, when mice were irradiated with 600- to 800-rad X rays and various tissues were then analyzed, we found an increase in the enzyme in the spleen at 2 days after exposure.

REFERENCE

1. Dobbs, C. R., Elhardt, C. E., Kumar, K. S., Weiss, J. F., and Catravas, G. N. Generation of N-pentane and alteration in the microheterogeneity of proteins on irradiation of microsomes. In: Abstracts of Papers, 178th National Meeting. American Chemical Society, Washington, D.C., 1979, Abstract no. BIOL-69.

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EXPERIMENTAL HEMATOLOGY DEPARTMENT

Exposure to ionizing radiation doses of 100-200 rads will damage or destroy bone marrow cells, resulting in reduction of or cessation in production of granulocytes, macrophages, and platelets, which are the first and major defense against infectious bacteria and their toxins. With increased radiation doses above 200 rads, these infections result in fatalities. Infections and fatalities can be decreased by procedures that protect bone marrow cells from these effects, or that enhance their endogenous production postirradiation, or that temporarily supply functional granulocytes until the radiation-damaged bone marrow recovers. In addition, successful treatment is promoted by means that would prevent the invasion of intestinal gram-negative bacteria into other tissues and organs of irradiated persons or at least reduce the concentration of those bacteria. Successful treatment would permit (a) the exposure of persons to higher radiation doses if demanded by extreme military situations and (b) the use of enhanced nuclear weapons since the treatment would raise the radiation dose that would cause 5% fatalities. Studies are also conducted to (a) develop therapy for damage from exposures to higher radiation doses that do not completely destroy bone marrow stem cells and (b) develop replacement of bone marrow. In addition, one important project deals with the complex effects of multiple injuries, and another deals with the possible late effects in survivors of low doses of radiation.

The Departmental program, Postirradiation Protection From Fatal Infections, is divided into six project groups, each researching a specific area.

PROJECT GROUP 1: Enhancement of White Cell Production Postirradiation

These projects are to elucidate the interaction of (a) the humoral substances released by functional white blood cells in loci of inflammation and infection and (b) the primitive precursor cells for increased production of adult functional cells. Of particular interest is the task of learning how to manipulate the radiation-injured precursor system by molecular engineering in order to enhance the production of white blood cells to fight against invading bacteria and their toxins. Significant progress has been made in the determination of humoral and cellular interactions, the proliferation capabilities of postirradiation stem and precursor cells, and the relative biological effectiveness of neutron radiation to these cells.

PROJECT GROUP 2: Studies of Origin and Prevention of Infection Postirradiation

These projects deal with experimental designs to discover possible routes of bacterial invasion postirradiation, means of preventing this occurrence, and means of increasing defense against the bacteria and their toxins in a radiation-injured organism. Studies were completed on the effects of Corynebacterium parvum and on the postirradiation disruption of intestinal tight junctions, normally a defense against intestinal bacteria and their toxins.

PROJECT GROUP 3: Combined Injury

Military analysts have estimated that, in a future atomic war, more than 70% of the casualties will suffer certain injuries in addition to those caused by ionizing radiation. The greater percentage of those injuries will be wounds or burns. German and Russian studies using mice or dogs indicate that the presence of open wounds after radiation will increase the number of fatalities whereas the immediate suturing of open wounds will not. Unfortunately, because of septic conditions, surgeons usually postpone the suturing of wounds

in military field conditions. Since the hematopoietic system is involved in the healing of wounds, it is important to study that system's functional status in the irradiated animal. Studies to date point out that the incidence of survival is affected by both the size of wounds and the timing of trauma in relation to exposure to radiation. Apparently the capacity of the lymphomyelopoietic system to withstand a second series of injuries is compromised.

PROJECT GROUP 4: Physiological Assessment of Fresh and Cryopreserved Granulocytes and Macrophages Used for Postirradiation Transfusion

Bone marrow exposed to radiation doses of 350-500 rads still has the capability of recovering if the animal or human does not die from infection. The best treatment is the infusion of compatible granulocytes. Methodology for the isolation of granulocytes by counterflow centrifugation-elutriation (CCE) was continued and improved. We demonstrated that the principles of CCE can be extended to an enlarged separation chamber for the isolation of therapeutic numbers of highly purified granulocytes for animal-model transfusion studies. We also showed that CCE-isolated canine granulocytes maintained both in vitro and in vivo efficacy without demonstrable loss of physiological function as a result of the dual leukapheresis and CCE-isolation procedure. In a separate study on the physiological function of macrophages, we observed that these cells exhibit complex electrophysiological properties often associated with excitable cells.

PROJECT GROUP 5: Transplantation of Bone Marrow Cells Into Lethally Irradiated Animals

Once radiation completely destroys the bone marrow, no endogenous recovery is possible. In such a case, transplantation of bone marrow between genetically identical persons is the only means of treatment and recovery. However, genetically identical cells usually are not available (with exception of those from identical twins), and the transplantation of incompatible bone marrow results in death. A study was conducted to assess the capability of compatible bone marrow to rescue mice from an LD50 dose (lethal dose for 50% of subjects) of neutron or gamma irradiation. We determined that a significantly greater concentration of bone marrow stem cells was needed to rescue mice after neutron irradiation than after gamma irradiation.

PROJECT GROUP 6: Late Effects of Ionizing Radiation

In recent years, the possibility has become apparent that military personnel exposed to very low doses of radiation may show an increase of degenerative diseases years later. To obtain greater insight into this phenomenon, the studies in this project group were initiated.

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PROJECT GROUP 1

STUDY OF RELATIONSHIP OF PROLIFERATION OF HEMOPOIETIC STEM CELLS TO SURVIVAL AFTER EXPOSURE TO IONIZING RADIATION

Principal Investigator: M. P. Hagan
Collaborator: T. J. MacVittie
Technical Assistance: D. P. Dodgen and R. T. Brandenburg

The kinetics of control of proliferation for the hemopoietic stem cell and its progeny has been partially established using continuous infusion of BrdUrd in vivo and using near-ultraviolet (NUV) light irradiation in vitro. The time-integrated S-phase fraction of the murine spleen colony-forming unit (CFUs) has been measured. These measurements indicate a stochastic commitment to cycle of essentially the entire population of CFUs in the steady state, consistent with the existence of a noncycling cell state. The data indicate that for B6D2F1 female mice, the CFUs are committed to deoxyribonucleic acid (DNA) synthesis approximately once every 52 hours. In addition, measurement of other CFUs parameters showed no significant effects of the BrdUrd-measuring technique itself. Challenge of BrdUrd-infused mice with hydroxyurea indicates that the capacity of CFUs to respond to a proliferative stimulus during the assay period is also unaffected.

This technique for analyzing kinetics has also been extended to the agar culture colony-forming unit (GM-CFUc). The single-cell survival curves after NUV light for BrdUrd-labeled GM-CFUc are composed of two components with widely disparate parameters of survival curve. As the BrdUrd infusion period is increased, the less sensitive surviving population becomes progressively sensitized. However, the ratio of the two population sizes remains essentially unchanged throughout the 7 days of the infusion period. More importantly, the NUV-light-resistant population becomes more sensitive, with kinetics identical to that of the CFUs. Unlike the resistant CFUs population, which bears no detectable BrdUrd label, the NUV-light-resistant GM-CFUc population has replicated at least once in the presence of BrdUrd. These data are consistent with the notion that the NUV-light-resistant population of GM-CFUc has arisen from recently divided CFUs.

The two efforts described above represent the proliferation model for the study of hemopoietic recovery after ionizing radiation.

Initial radiation experiments for this model have shown increases in CFUs proliferation at doses as low as 40 rads. This increased proliferation is dramatic on the first day after exposure to radiation. Developing this observation will be the objective of work within the next fiscal year.

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DETECTION OF GAP JUNCTIONS BETWEEN THE PROGENY OF A CANINE MACROPHAGE COLONY-FORMING CELL *IN VITRO*

Principal Investigators: M. Porvaznik and T. J. MacVittie
Technical Assistance: J. L. Atkinson, R. T. Brandenburg, and E. G. McCarthy

An in vitro monocyte-macrophage colony-forming cell (M-CFC) has been detected in canine bone marrow. The colonies derived from these progenitor cells were similar to murine-derived M-CFC colonies (1), since (a) they showed a singular macrophage line of differentiation and a lag of 14-16 days before initiating colony formation, and (b) they survived significantly longer in culture in the absence of colony-stimulating factor than did granulocyte-macrophage colony-forming cells (GM-CFC) (Figure 1).

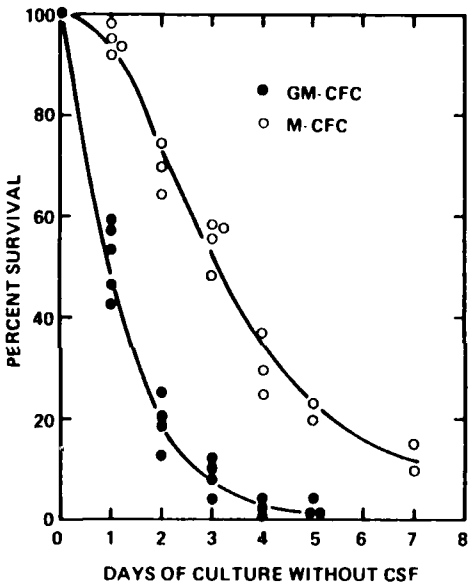

Figure 1. Surviving fraction of colony-forming cells (GM-CFC, M-CFC) derived from bone marrow cells versus time in culture in absence of colony-stimulating factor. Values represent means of separate experiments.

Dog serum stimulated with endotoxin (Salmonella typhosa lipopolysaccharide W) was used as the colony-stimulating factor (7% volume for volume). Canine-derived M-CFC progeny were identified as macrophages on the basis of morphology, phagocytosis, and the presence of Fc receptors for IgG. Using freeze-fracture and lanthanum tracer techniques, gap junctions were observed only in colonies derived from canine bone marrow M-CFC. The gap junctions were not observed in any GM-CFC-derived colonies. The number of gap junctions observed in freeze-fracture replicas of bone marrow M-CFC-derived colonies (21 colonies from three different dogs) showed a significantly positive correlation (Kendall = 0.70, $p = 0.001$) to the size of the fracture plane area through a colony. Gap junctions were observed displaying hexagonal lattices of 9.3 nm $\pm$ 0.08 (SE) particles with a center-to-center spacing of 10.4 nm $\pm$ 1.0 (SE) on membrane P-fracture faces. On membrane E-fracture faces, highly ordered arrays of pits with 8.7 nm $\pm$ 0.12 (SE) center-to-center spacing were observed. Arrays of both particles and pits were also observed in fracture-face breakthroughs within a gap junction.

Thus, gap junctions can form in vitro between the cells of macrophage progeny of a canine M-CFC under appropriate conditions of growth.

The significance of this observation is that a structural basis may exist for cell-to-cell collaboration between macrophages and other capable cells that either pass into the tissue for modification or develop there into mature cell forms.

REFERENCE

1. MacVittie, T. J. and Porvaznik, M. Detection of in vitro macrophage colony-forming cells (M-CFC) in murine bone marrow, spleen, and peripheral blood. Journal of Cellular Physiology 97: 305-314, 1978.

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RADIOSENSITIVITY AND RELATIVE BIOLOGICAL EFFECTIVENESS OF HEMATOPOIETIC PLURIPOTENT AND COMMITTED STEM CELLS TO COBALT-60-GAMMA AND/OR FISSION NEUTRONS FROM THE AFRRI TRIGA MARK-F REACTOR

Principal Investigators: R. M. Vignuelle, S. J. Baum, and S. R. Weinberg

The radiosensitivity and the relative biological effectiveness of pluripotent stem cells and some committed progenitor cells derived from bone marrow and/or spleen of an inbred mouse (the National Institutes of Health Swiss white) were measured. The measurements were made using in vivo and/or in vitro assays for the colony-forming unit-spleen (CFU-S), the colony-forming unit-erythroid (CFU-E), the granulocyte-macrophage colony-forming cell (GM-CFC), and the macrophage colony-forming cell (M-CFC).

The radiosensitivity was determined by sacrificing groups of mice at 24 hours after either (a) a bilateral exposure to 0.5, 1.0, 2.0, 3.0, and 4.0 grays of cobalt-60-gamma rays at a dose rate of 0.4 grays/min or (b) 0.5, 1.0, 1.5, and 2.0 grays kerma (free-in-air) of fission neutrons from the AFRRI TRIGA Mark-F Reactor. Pairs of mice, individually caged in aluminum holders, were rotated on a vertical axis in a relatively gamma-free field. The field was obtained by exposing the mice within a lead cave (2 inches thick) behind a lead shadow shield (6 inches thick) and a gadolinium/cadmium absorber for thermal neutrons.

A dose-related decrease in pluripotent and committed stem cells was found in both the bone marrow and the spleen after exposure to either cobalt-60-gamma rays or fission neutrons. The radiosensitivity of bone marrow-deprived pluripotent and committed stem cells was lower after exposure to fission neutrons than after exposure to cobalt-60-gamma rays. A similar result was observed for the spleen. The spleen-derived CFU-S and CFU-E were more radiosensitive than those derived from the bone marrow after either cobalt-60-gamma rays or fission neutrons. Estimates of the radiosensitivity of GM-CFC or M-CFC did not differ

between bone marrow or spleen with either kind of radiation. The relative biological effectiveness (cobalt-60-gamma/fission neutron) is 2.2 for each assay of hematopoietic stem cells measured. The relative biological effectiveness that is determined using hematopoietic stem cells derived from bone marrow differs little from that determined using the spleen.

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DETECTION OF *IN VITRO* MACROPHAGE COLONY-FORMING CELLS IN MOUSE BONE MARROW, SPLEEN, AND PERIPHERAL BLOOD

Principal Investigators: T. J. MacVittie and M. Porvaznik

Technical Assistance: E. G. McCarthy, R. G. Mitchell, R. T. Brandenburg, and J. L. Atkinson

In vitro macrophage colony-forming cells (M-CFC) have been detected in bone marrow ($317/10^5$ cells), spleen ($81/10^5$), and peripheral blood leukocytes ($242/10^5$) of the mouse. These M-CFC were similar to those previously detected in thymus tissue ($30/10^6$) and lymph node tissue ($22/10^6$) in several respects (Table 1).

Table 1. Frequency and Clusters-to-Colony Ratios of Colony-Forming Cells (M-CFC, CFU-c) from Various Hematopoietic Sites in the Mouse

	Macrophage colony forming cells (M-CFC, 25-30d)			
	M-CFC/10^5 cells	M-CFC/organ	Cluster/M-CFC ratio	
Bone marrow, femur	3,170 ± 40	61,058 ± 12,161	2.4	(12)
Spleen	811 ± 135	122,590 ± 24,650	2.8	(15)
Peripheral blood mononuclear cells	2,420 ± 627	3,252 ± 475	0.8	(11)
Thymus	28 ± 3.7	2,449 ± 425	0.8	(9)
Lymph node, cervical	28 ± 2.5	929 ± 90	1.3	(9)
	Granulocyte-macrophage colony forming cells (CFU-c, 7-10d)			
	CFU-c/10^5 cells	CFU-c/organ	Cluster/CFU-c ratio	
Bone marrow, femur	2,350 ± 108	44,928 ± 3,710	6.4	(12)
Spleen	50 ± 8	8,626 ± 1,070	3.6	(12)
Peripheral blood mononuclear cells	53 ± 10	48 ± 11	10.8	(12)

[illegible] ± SEM. M-CFC per organ [illegible] total nucleated cells per organ. [illegible] experiments.

Bone marrow-derived and spleen-derived M-CFC required pregnant mouse uterine extract to consistently initiate colony formation, whereas peripheral blood leukocyte-derived M-CFC formed colonies with stimulation by either pregnant mouse uterine extract or L-cell-conditioned medium. All formed colonies showed a singular macrophage line of differentiation, a lag of 13-18 days before initiation of colony formation (Figure 1), a marked ability to survive in culture in the

absence of pregnant mouse uterine extract (Figure 2), and markedly slow rates of appearance in culture once colony formation was initiated.

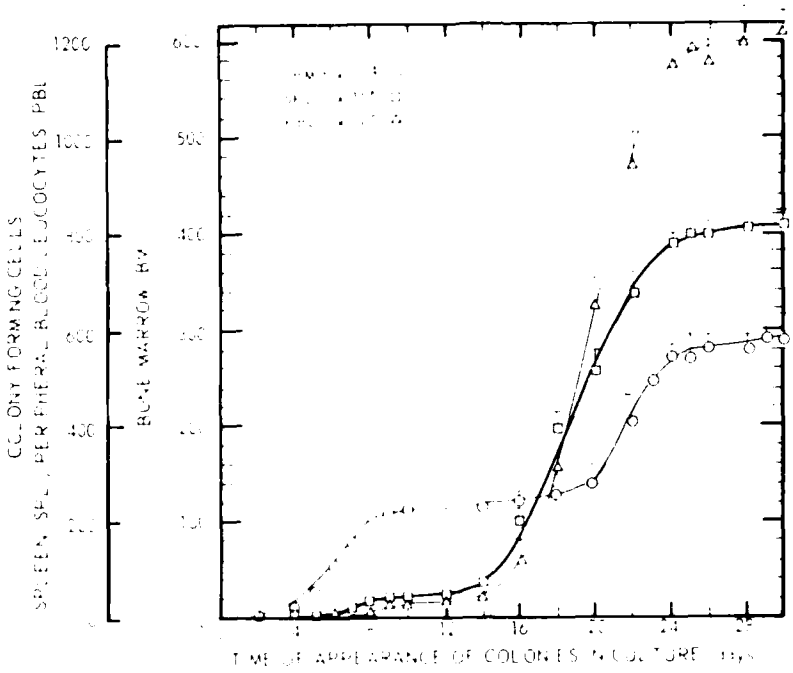

Figure 1. Appearance of colonies derived from bone marrow ( ) 5×10^4 cells, spleen ( ) 1×10^6 cells, and peripheral blood mononuclear ( ) 5×10^5 cells cultured as a function of time. Mean values (±SEM) are from four replicate experiments.

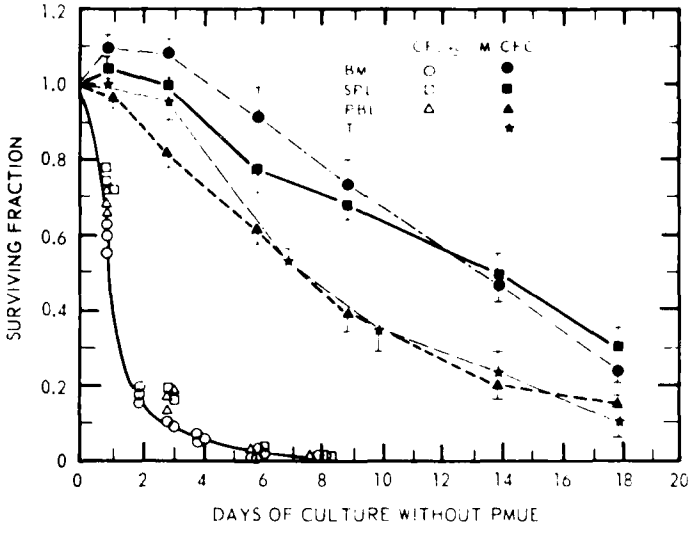

Figure 2. Surviving fraction of colony-forming cells (CFU-c, M-CFC) derived from bone marrow (, ●), spleen (, ■), peripheral blood mononuclear cells (, ▲) and thymus (*) versus time in culture in absence of PMUE. Values are means (± SEM) from three replicate experiments.

The macrophage progeny were identified on the basis of morphology; adherence to glass; the phagocytosis of agar, bacteria, and sheep red blood cells; and the presence of receptors for IgG.

These characteristics are also shared by those macrophge colony-forming cells observed within stimulated peritoneal exudate, pleural effusion, and alveolar space. These M-CFC are most likely members of a large, heterogeneous population of macrophage progenitor cells distributed throughout the hemato-lymphopoietic organs, serosal cavities and surfaces, and inflammatory and alveolar tissue sites. The degree of heterogeneity may be determined in part by the influence of tissue-specific microenvironment.

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PROJECT GROUP 2

ALTERATIONS INDUCED IN MURINE HEMOPOIETIC STEM CELLS AFTER A SINGLE INJECTION OF *CORYNEBACTERIUM PARVUM*

Principal Investigator: T. J. MacVittie
Technical Assistance: E. G. McCarthy, R. G. Mitchell, R. T. Brandenburg, and J. L. Atkinson

A single injection of Corynebacterium parvum into normal mice resulted in marked alterations within the hemopoietic stem cell (CFU-s) populations as measured by both exogenous and endogenous techniques. Exogenous stem cell content was determined for bone marrow, spleen, and peripheral blood. In addition, we measured the fraction of marrow and spleen CFU-s in cell cycle and their seeding efficiencies.

After an injection of mice with C. parvum, there was an initial differential depletion of non-CFU-s within the bone marrow. This resulted in a twofold increase in CFU-s concentration and the consequent maintenance of normal CFU-s content during the 1st week (Table 1). During this period, marrow CFU-s entered cell cycle, their seeding efficiency decreased, and a certain fraction was mobilized into the peripheral circulation. The CFU-s in the circulation peaked at 3 days and then decreased to a value approximately threefold of control by day 7. This elevated level was maintained in the circulation throughout the next 2 weeks (Figure 1).

Table 1. Concentration of Exogenous Stem Cells[a] in Bone Marrow, Spleen, and Peripheral Blood Leukocytes and the Endogenous Stem Cell[b] Response Following Injection of *Corynebacterium parvum* (6134)

		DAYS AFTER *C. PARVUM*								
Stem Cells	Control	1	2	3	4	7	10	14	17	21
Exogenous										
Bone marrow	35.5 ± 2.2	48.9 ± 3.9[c]	69.3 ± 10.3[c]	69.2 ± 5.6[c]	41.7 ± 3.4	36.2 ± 6.8	31.2 ± 5.1	35.8 ± 2.4	44.0 ± 4.6	57.5 ± 0.8[c]
Spleen	3.2 ± 0.7	8.2 ± 1.7[c]	9.7 ± 1.2[c]	15.8 ± 3.7[c]	19.3 ± 3.1[c]	20.2 ± 6.8[c]	9.2 ± 2.5[c]	3.8 ± 0.6	5.2 ± 0.9	7.8 ± 0.5
Peripheral blood leukocyte	0.9 ± 0.5	1.8 ± 0.3	3.9 ± 0.8[c]	4.2 ± 0.6[c]	2.5 ± 0.3	1.7 ± 0.1	1.2 ± 0.4	1.9 ± 0.2	0.8 ± 0.1	1.46 ± 0.1
Endogenous	4.3 ± 0.55	23.1 ± 2.0[c]	37.4 ± 6.2[c]	32.1 ± 4.2[c]	27.3 ± 3.7[c]	34.5 ± 7.1[c]	28.0 ± 5.0[c]	40.1 ± 4.7[c]	25.0 ± 6.0[c]	27.1 ± 3.1[c]
Spleen weight (mg)	26 ± 2.4	44 ± 3.6	106 ± 8.5	60 ± 5.1	62 ± 3.2	126 ± 6.1	127 ± 4.3	116 ± 5.7		

Exogenous values (± SEM) are means per 10^5 nucleated cells of at least four replicate experiments.
[c] Values are significantly different from control BM ($p = .01$ to $.0005$, SPL ($p = .05$ to $.005$, PBL ($p = .025$. Endogenous values are means (± SEM) (number of surface colonies per spleen, eight spleens per group) ($p = .0005$. Spleen weight equals mean value (± SEM) of endogenous spleens.

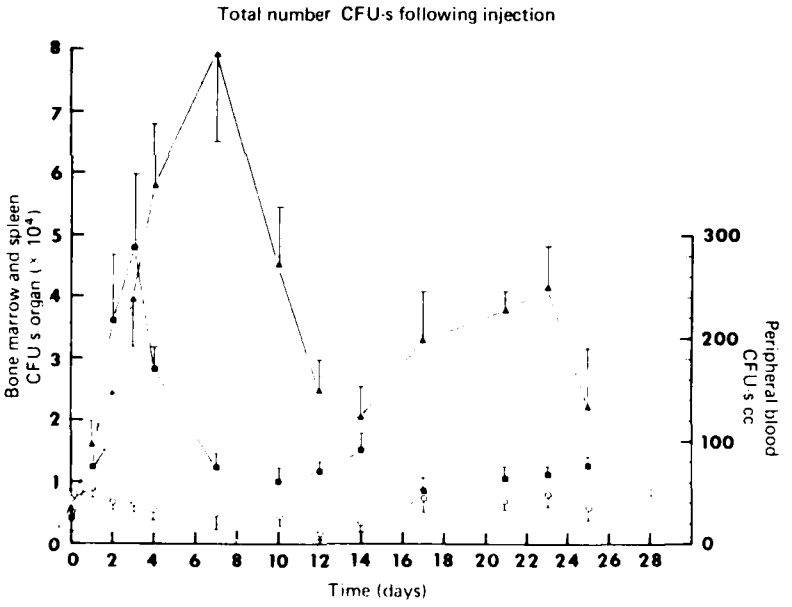

Figure 1. Number of CFU-s per femur and spleen and per milliliter peripheral blood at various times after injection of 1.4 mg of *Corynebacterium parvum* (6134) in mice. Values are means (± SEM) of at least four replicate experiments. Bone marrow (○—○), spleen (▲—▲), and peripheral blood (■—■).

The splenic content of CFU-s increased exponentially to peak values twelvefold greater than control by day 7 (Figure 1). As with the marrow, a large number of splenic CFU-s had entered the cycle by day 3 and remained in cycle at day 7; they had a significantly decreased seeding efficiency. Stem cell content decreased over the 2nd week and then peaked again over the 3rd week to values sixfold greater than control (Figure 1). The increased stem cell activity was associated with a significant increase in spleen size and a marked five- to eightfold increase in endogenous colony formation from day 1 through day 21 (Table 1). These results indicated an intense and prolonged effect of C. parvum on the hemopoietic stem cell populations.

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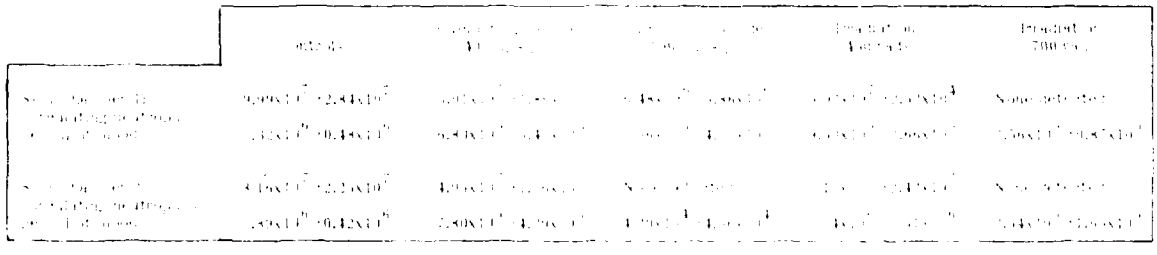
EFFECTS OF IRRADIATION OR CYCLOPHOSPHAMIDE ON LOCAL LEUKOCYTE MOBILIZATION IN RATS

Principal Investigators: B. H. Gray and W. H. Baker
Technical Assistance: D. Walden

Local leukocyte mobilization (LLM) is the migration of neutrophils to a skin abrasion site assayed by gluing a flexible plastic cup over the site and counting trapped cells at 24 hours after treatment (1). Neutrophil migration to an infection site is a primary event in the inflammatory response, and reduction of an animal's capacity to mount this response may contribute to mortality after irradiation.

The LLM response was assayed in female Sprague-Dawley rats (weighing 250-325 g) after exposure of the animals to cobalt-60 radiation or cyclophosphamide. The LLM was determined in treated rats when the blood-circulating neutrophil count had reached a nadir at 3 or 4 days after treatment. Skin chambers on one lateral surface were quantitated, and then a second chamber was mounted over a second lateral abrasion to yield the LLM for the second chamber. Results of these studies are summarized in Table 1.

Table 1. Local Leukocyte Mobilization* in Rats Either Treated With Cyclophosphamide or Irradiated With Cobalt-60



Magnitude of the LLM response depended on the number of circulating neutrophils in the rats. Animals exposed to 450 rads of cobalt-60 or 40 mg/kg cyclophosphamide had an LLM response. Even 100 mg/kg cyclophosphamide was insufficient to block neutrophil migration the first day the LLM was assayed. However, 700 rads of cobalt-60 exposure eliminated the LLM response even though blood-circulating neutrophils were in the 10^5 per ml range. Neutrophils in such animals may have a reduced ability to migrate, marginate, or undergo chemotaxis.

REFERENCE

1. Senn, H. J. and Holland, J. F. Leukocyte mobilization in health and acute leukemia. Blood 30: 888, 1967.

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TIGHT JUNCTION DISRUPTION AND RECOVERY AFTER SUBLETHAL IRRADIATION

Principal Investigator: M. Porvaznik

Ilea from sublethally irradiated, adult rats were prepared for freeze-fracturing and lanthanum tracer study to investigate the alterations that occur in the structure and function of the intestinal permeability barrier.

Some of the tight junction structures were determined to be focally disrupted between days 1 and 5 postirradiation using the freeze-fracture technique. Alterations in mean depth of the apical tight junction appeared to correlate with permeability of the epithelium to lanthanum tracer (Figure 1). Occurrence of

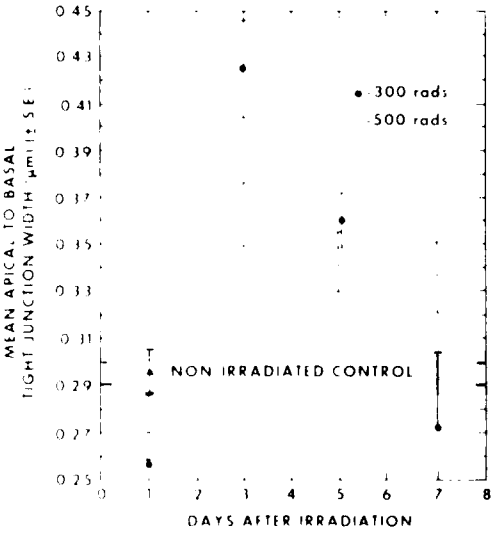

Figure 1. Time course of change [illegible] tight junctional complex in apical [illegible] meters (± SE). These observations [illegible] measurements from every tight junction [illegible] 300 rad (●) or after 500 rad (○). Range [illegible] measurements is denoted by shaded zone.

tight junctional fragments over extensive areas between lateral membrane fracture faces were observed during the disruption and recovery phases. They were manifest as linear and macular tight junctions that extended basally from the apical tight junction (zonula occludens) as far as the basal lamina. These "proliferative" tight junction fragments were thought to be eventually removed by phagocytosis since numerous tight junctional fragments could be observed in cytoplasmic vesicles between days 3 and 7 after irradiation.

Lanthanum tracer, added only to the intestinal lumen during fixation for electron microscopy, was found in extracellular spaces between some goblet cells and adjacent absorptive epithelial cells in preparations from irradiated rats. "Leaky" tight junctions were observed between days 1 and 7 postirradiation; then they returned to control levels.

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PROJECT GROUP 3

SURVIVAL OF MICE AFTER SKIN WOUND TRAUMA AND IRRADIATION

Principal Investigators: G. D. Ledney and D. A. Stewart
Technical Assistance: E. D. Exum

Effects of nuclear weapons include the biological damage induced by various qualities of radiation, by burn, and by mechanical traumas. We determined the survival of mice after various sizes of wounds given either alone or in connection with a sublethal dose of cobalt-60 radiation.

Two replicate experiments were performed as recorded in Table 1. Adult CBA male mice were anesthetized with metaphane and given skin wounds in the anterior dorsum. The wounds were not treated with antibiotics, and they remained open to the cage environment. Sizes of skin wounds had been previously determined by assessing the portion of skin removed versus the total skin surface area of the mouse. Survival after skin wounding alone, irradiation alone, and skin wounding combined with irradiation were recorded over an observation period of 30 days.

Generally, only a few mice died after a loss of as much as 8% of the total skin surface area. About 50% of the mice died after exposure to 650 rads cobalt-60. Mice given a skin wound of either 2% or 4% body surface either before or after 650 rads radiation survived either as well as or better than those mice given only radiation. Deaths occurring in mice wounded after irradiation were associated with septicemia. Essentially, all mice given an 8% wound before or after sublethal irradiation died. This points to a limit in the amount of damage that a host can incur from two sublethal events.

Table 1. Survival of Mice After Radiation and Wound Trauma

Experimental Treatment	First-Set Injuries		Second-Set* Injuries	
	Survival Fraction	MST ± SE	Survival Fraction	MST ± SE
2% wound 24 h pre 650 rad	18/20	9	17/18	13
2% wound 1 h pre 650 rad	18/20	9.5 ± 0.7	18/18	-
2% wound 1 h post 650 rad	17/20	11.7 ± 2.1	17/17	-
2% wound 24 h post 650 rad	17/19‡	12.0 ± 2.8	14/17	9.3 ± 3.1‡
Only 2% wound	19/20	3	19/19	-
4% wound 24 h pre 650 rad	16/20	15.0 ± 3.5	12/16	11.5 ± 5.0
4% wound 1 h pre 650 rad	12/20	18.4 ± 5.0	11/12	10
4% wound 1 h post 650 rad	18/20	11.0 ± 2.8	14/18	12.5 ± 3.3
4% wound 24 h post 650 rad	17/19‡	13.0 ± 5.7	8/15‡	9.7 ± 1.3‡
Only 4% wound	18/20	15.0 ± 8.5	18/18	-
8% wound 24 h pre 650 rad	1/10	14.2 ± 4.8	N.D.§	-
8% wound 24 h post 650 rad	0/10	9.1 ± 2.6	N.D.	-
Only 8% wound	9/10	14	N.D.	-
Only 650 rad	12/20	13.9 ± 6.4	12/12	-
Untreated control	5/5	-	5/5	-

* Mice alive 60 days after first set of injuries were given a second similar set of injuries.

‡ Mean survival time

‡ Mice died from septicemia.

§ ND = not done.

All mice that survived the initial radiation and wounding experiments were subjected to a second set of similar injuries 60 days later. A second wound or similar dose of radiation did not result in any 30-day mortality. Generally, a greater incidence of mortality was seen in all mice wounded after exposure to radiation (20%) than in mice wounded before exposure to 650 rads (10%). Also, a greater incidence of mortality was seen for all mice given a 4%-body surface skin wound and sublethal radiation (25%) than for mice given the smallest wound in combination with radiation (5%).

This investigation points out that the incidence of survival is affected by both the size and the timing of the wound in relation to the exposure to radiation. Further, the capacity of the lymphomyelopoietic system to withstand a second series of injuries seems compromised.

PROJECT GROUP 4

LIQUID STORAGE AND CRYOPRESERVATION STUDIES OF PURIFIED GRANULOCYTES ISOLATED BY COUNTERFLOW CENTRIFUGATION-ELUTRIATION

Principal Investigators: J. F. Jemionek and T. J. Contreras
Technical Assistance: D. Walden

The transfusion of leukocytes is an adjunct to the use of antibiotics in treating sepsis in radiation-induced or drug-induced neutropenic patients. The clinical benefits are well documented in the literature. The common methods of isolating leukocytes have been filtration leukapheresis, continuous-flow centrifugation leukapheresis, and discontinuous-flow centrifugation leukapheresis. These systems have permitted the isolation of large numbers of leukocytes (8×10^9 to 3×10^{10}) containing significant numbers of granulocytes (PMN) (5×10^9 to 2×10^{10}) for transfusion therapy.

Counterflow centrifugation-elutriation (CCE) is another method of isolating polymorphonuclear leukocytes, which is used primarily as a research tool for preparing highly purified polymorphonuclear leukocytes (greater than 95%) from small quantities of peripheral blood. We have postulated that due to the purity of human polymorphonuclear leukocyte recovered, the principles of CCE, if applied to the isolation of human leukocytes, may supply a blood product for therapy by transfusion of human polymorphonuclear leukocytes that is better than the blood product currently used (1). We have previously reported the efficient isolation by CCE of $2.8\text{-}5.0 \times 10^8$ polymorphonuclear leukocytes from 120 ml of peripheral human or canine blood and the recovery of $1.0\text{-}1.3 \times 10^9$ polymorphonuclear leukocytes from small quantities of leukapheresis human or canine concentrate obtained by continuous-flow centrifugation-leukapheresis (2). We also demonstrated that CCE-isolated polymorphonuclear leukocytes displayed no apparent physiological damage as a result of the isolation procedure, based on in vitro analysis of viability, enzyme activity, electron microscopy, latex bead ingestion, bactericidal activity, and chemotactic response (3).

However, in vitro data do not predict the ability of these cells, once returned in vivo, to retain physiological function. In order to conduct in vivo analysis of CCE-purified polymorphonuclear leukocytes in animal models, sufficiently large numbers of polymorphonuclear leukocytes are required. The ability to achieve enhanced recovery of polymorphonuclear leukocytes necessarily requires modification of the Beckman Instruments JE6 rotor. This modification depends ultimately on directly enlarging the isolation chamber or modifying the design of the entire rotor system.

The results of our investigation were twofold: First, we demonstrated that the principles of CCE can be extended to an enlarged separation chamber for the isolation of therapeutic numbers of highly purified polymorphonuclear leukocytes

for transfusion studies in an animal model. Second, we showed that CCE-isolated canine polymorphonuclear leukocytes maintained both in vitro and in vivo efficacy without demonstrable loss of physiological function as a result of the dual leukapheresis and CCE isolation procedure.

REFERENCES

1. Contreras, T. J., Jemionek, J. F., French, J. E., and Hartwig, V. Liquid preservation of canine granulocytes obtained by counterflow centrifugation-elutriation. Experimental Hematology 6: 767-776, 1978.

2. Jemionek, J. F., Contreras, T. J., French, J. E., and Shields, L. J. Granulocyte isolation by counterflow centrifugation-elutriation of canine blood obtained by continuous-flow centrifugation leukapheresis. Experimental Hematology 6: 801-808, 1978.

3. Jemionek, J. F., Contreras, T. J., French, J. E., and Shields, L. J. Technique for increased granulocyte recovery from human whole blood by counterflow centriguation elutriation. I. In vitro analyses. Transfusion 19: 120-128, 1979.

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FRACTIONATION OF CANINE AND HUMAN BONE MARROW ASPIRATES BY COUNTERFLOW CENTRIFUGATION-ELUTRIATION FOR ANALYSIS OF GM-CFC ACTIVITY

Principal Investigators: J. F. Jemionek and T. J. MacVittie
Technical Assistance: D. Walden

Fractionation of canine and human bone marrow aspirates by counterflow centrifugation-elutriation (CCE) was used to isolate granulocyte-macrophage colony-forming cell activity (GM-CFC). Cell separation was achieved by varying the viscosity of the elutriation medium from a specific gravity of 1.0123 to 1.0394, using Cohn Fraction V bovine serum albumin 2-14 gm% (weight per volume) in phosphate-buffered saline. The density gradient for elutriation was established using an LKB Ultragrad gradient instrument.

Canine bone marrow aspirates pretreated with Ficoll-Hypaque to remove erythrocytes displayed a cell separation profile different from nontreated canine bone marrow used for CCE. Canine bone marrow pretreated with Ficoll-Hypaque displayed a peak of GM-CFC in fractions 3-5, which corresponds to an eluate specific gravity of 1.0168-1.0216. Nontreated canine bone marrow displayed two peaks of GM-CFC activity. The first was in fraction 2, which corresponds to an eluate specific gravity of 1.0123, and the second peak occurred in fractions 7-9, which corresponds to an eluate specific gravity of 1.0213-1.0262. Human bone

marrow aspirates obtained through a collaborative study with Georgetown University Medical Center were not treated before CCE. Variations in GM-CFC profiles were observed. When the marrow tap was a single tap in which significant blood with few spicules comprised the aspirate sample, then a biphasic peak of GM-CFC activity was obtained at fractions 6-14, which corresponds to an eluate specific gravity at 1.0181-1.0357. When the bone marrow aspirate was a multiple tap in which significant numbers of bone spicules were present, then two areas of peak GM-CFC activity were observed. The first peak was obtained in fraction 2, which corresponds to an eluate specific gravity of 1.0123. The second peak GM-CFC was a biphasic peak that occurred in fractions 6-14, or an eluate specific gravity of 1.0181-1.0357.

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NONLINEAR CURRENT-VOLTAGE RELATIONSHIPS IN CULTURED MACROPHAGES

Principal Investigators: E. K. Gallin and D. R. Livengood
Technical Assistance: D. Darden

Intracellular recordings of cultured mouse thioglycollate-induced peritoneal exudate macrophages reveal that these cells can exhibit two different types of electrophysiological properties characterized by differences in their current-voltage relationships and their resting membrane potentials (1).

Figure 1 shows the distribution of resting membrane potentials in these cultured macrophages. The majority of cells had low resting membrane potentials (-20 to -40 mV), and displayed current-voltage relationships that were linear for inward current pulses and rectifying for outward pulses. Small depolarizing transients, occurring either spontaneously or induced by current pulses, were seen in some cells with low resting membrane potentials. A second smaller group of cells exhibited more hyperpolarized resting membrane potentials (-60 to -90 mV) and S-shaped current-voltage relationships associated with a high-resistance transitional region. Cells with S-shaped current-voltage relationships sometimes exhibited two stable states of membrane potential on either side of the high-resistance transitional region.

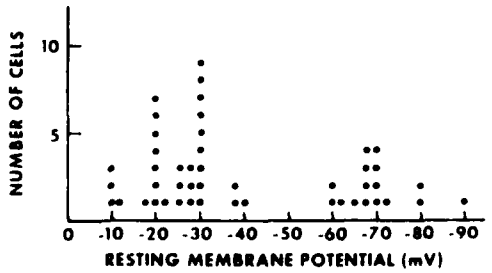

Figure 1. Mouse macrophages in HEPES-Hanks' solution. Figure shows distribution of resting membrane potentials from mouse peritoneal exudate macrophages cultured for 2-4 wk. Total number of cells

These data indicate that macrophages exhibit complex electrophysiological properties often associated with excitable cells.

REFERENCE

1. Gallin, E. K. and Livengood, D. R. Nonlinearity of the current-voltage relationship in cultured macrophages. Journal of Cell Biology, in press.

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PROJECT GROUP 5

$LD_{50/30}$ OF B6CBF1 MALE MICE EXPOSED TO MODIFIED NEUTRON FIELD WITHIN AFRRI TRIGA REACTOR

Principal Investigators: W. H. Baker and D. Stewart
Technical Assistance: E. D. Exum

Particularly important for understanding neutron radiobiology is the quantitation of gamma contamination in each exposure setting. Variations in physical design, experimental protocol, and the accompanying gamma contamination have contributed to the disparate 30-day survivals in previously published research (1-6) that used neutron sources. Parameters of radiation survival in a fission neutron field of B6CBF1 male mice (12-20 weeks old and weighing 25-?3 g) were investigated using a special exposure design within the AFRRI TRIGA Reactor. The design is capable of yielding a 30:1 neutron/gamma ratio (N/G) free in air and 14:1 N/G at depth (1.5), and consists of two lead shields that surround an aluminum rotator (Figures 1 and 2). The shields reduce the amount of gamma exposure from the reactor core (direct radiation) and from scatter produced in the exposure room (indirect radiation). The aluminum rotator, constructed of a low-alloy aluminum, reduces production of gamma within the lead cave.

Whole-body exposures were delivered at a free-in-air dose rate of 40 rads/min within the source field. The mice were rotated within the field at about 1.5 revolutions per min during the exposure to obtain a dose distribution and an N/G ratio in the animal as uniform as possible (7,8). The neutron survival results were compared to gamma survival data generated for the same mouse strain. Gamma exposures were made in the AFRRI cobalt source.

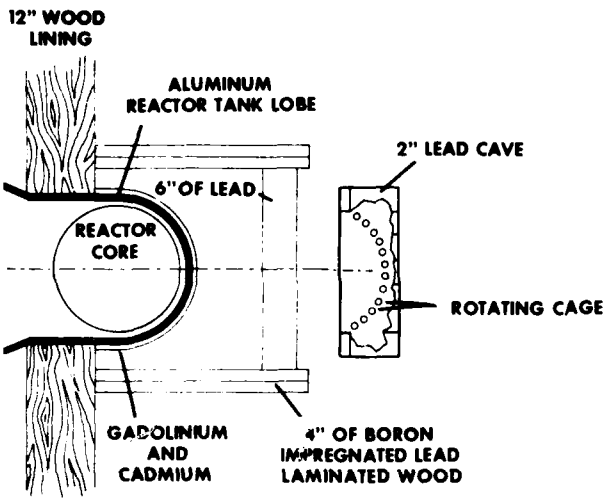

Figure 1. Exposure array for enhanced neutron field

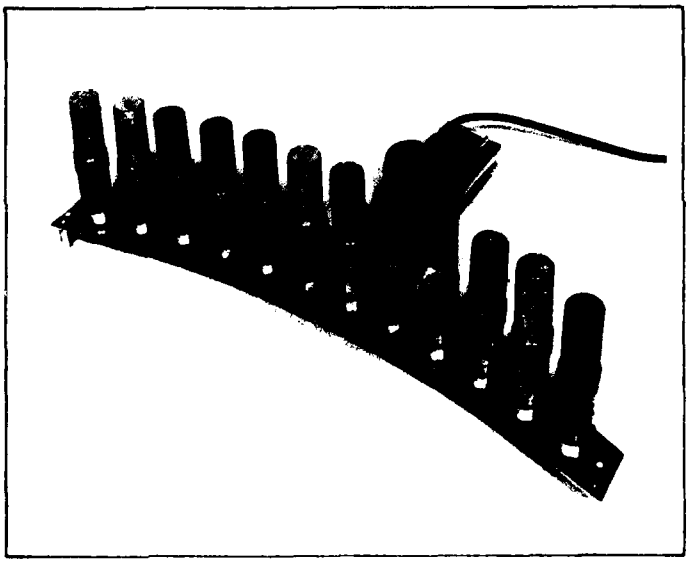

Figure 2. Aluminum rotator, constructed of low-alloy 6061 aluminum, rotates 20 mice at 1.5 revolutions per min.

The $LD_{50/30}$ values (lethal dose for 50% of animals after 30 days) for the neutron and the gamma irradiations were calculated to be 371 $\pm$ 4 rads and 879 $\pm$ 14 rads, respectively (Figure 3). The relative biological effectiveness of the neutrons was calculated from the probit plot for mortality to be 2.6 $\pm$ 0.05 at the $LD_{5/30}$, and ranged to 2.7 $\pm$ 0.03 at $LD_{95/30}$. The neutron curve shows a significantly steeper slope ($p = 0.001$), reflecting a much narrower dose range for lethality in the neutron-irradiated mice. These data are based on exposure runs displayed in Table 1.

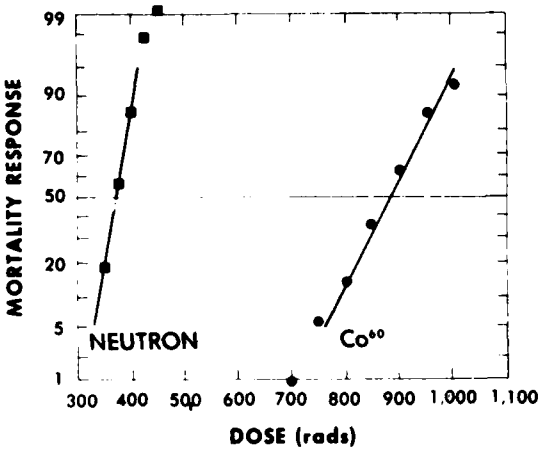

Figure 3. Dose response of B6CBF1 mice to 0.9-MeV fission neutrons (■) and cobalt-60-gamma rays (●).

Table 1. Mortality in Mice Exposed to 0.9-MeV Fission Neutrons and Cobalt-60 Gamma Rays

	Radiation dose (rads)	Number mice irradiated	Number mice dead within 30 days	Percent mortality
Neutron:				
(LD_{50} 371 ±4)	350	60	11	18
	375	130	68	52
	400	153	115	75
	425	180	168	93
	450	128	128	100
	475	53	53	100
Gamma ray:				
($LD_{50/30}$ 879 ± 14)	700	36	0	0
	750	36	2	6
	800	46	5	11
	850	36	13	36
	900	46	29	63
	950	36	31	86
	1000	40	37	93

Summation of the mortality distributions for successive neutron-dose intervals is diagrammed in Figure 4. The mean survival time (MST) is seen to shorten with increasing dose. The 8-day survival indicated by a vertical line in Figure 4 has been previously reported as the demarcation in time between death caused by gastrointestinal failure (gastrointestinal range) and death caused by hematopoietic failure (hematopoietic range).

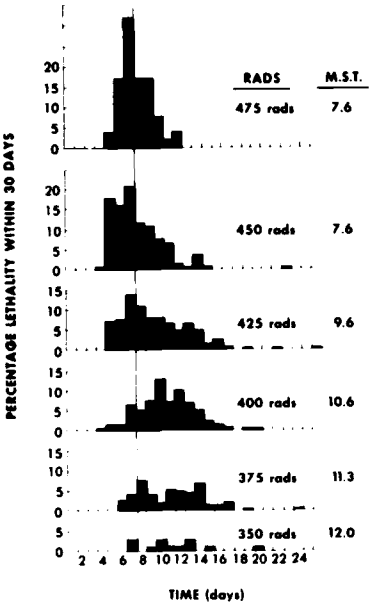

Figure 4. Mortality time distribution for B6CBF1 male mice as function of neutron dose. Line represents arbitrary division between gastrointestinal death and hematopoietic death.

This study demonstrates considerable differences in the response of the B6CBF1 mouse to modified fission neutrons and its response to cobalt-60-gamma rays. The fixed exposure rate of neutron irradiation was chosen to simplify the comparison of fission neutrons to gamma rays by eliminating the possible effects of dose rate. At identical dose rates of 40 rads/min, fission neutrons with an estimated average energy of 0.9 MeV exhibit a relative biological effectiveness of 2.36 ± 0.024 at $LD_{50/30}$. Further comparison of these mortality curves reveals a significant difference ($p = 0.001$) in the slopes of the curves. Disparity between the slopes is reflected in the dose range, which spans two biologically identifiable parameters, the $LD_{5/30}$ and $LD_{95/30}$. For gamma irradiation, the dose range from the onset of death to near 100% mortality is greater than 225 rads, whereas for fission neutrons it spans about 70 rads. This threefold difference substantiates the high rate of radiobiological damage suggested by the relative biological effectiveness calculated at $LD_{50/30}$.

Since these initial data on our array have been collected, the hematopoietic rescue of neutron-exposed mice will be investigated and compared to gamma recipients.

REFERENCES

1. Randolph, M. L., Congdon, C. C., Urso, I. S., and Parrish, D. L. Effect of bone marrow treatment on mortality of mice irradiated with fast neutrons. Science 125: 1083-1084, 1957.

2. Cole, L. J. and Ellis, M. E. Effect of bone marrow-spleen injection after fast neutron irradiation in mice. American Journal of Physiology 194: 16-22, 1958.

3. Alpen, E. L. and Baum, S. J. Autologous bone-marrow implantation after fast neutron irradiation of dogs. Radiation Research 11: 383-389, 1959.

4. Vogel, H. H., Jr. and Jordan, D. L. The combined action of bone marrow and streptomycin in protecting neutron-irradiated mice from acute mortality. Transplantation Bulletin 7: 413-415, 1960.

5. Davids, J. A. G. Bone-marrow syndrome in CBA mice exposed to fast neutrons of 1.0 MeV mean energy. Effect of syngeneic bone-marrow transplantation. International Journal of Radiation Biology 17: 173-185, 1970.

6. Broerse, J. J., Van Bekkum, D. W., Hollander, C. F., and Davids, J. A. G. Mortality of monkeys after exposure to fission neutrons and the effects of autologous bone marrow transplantation. International Journal of Radiation Biology 34: 253-264, 1978.

7. Rossi, H. H. and Goodman, L. J. Measurement of tissue dose of fast and slow neutrons. Annual Report on Research Project, NYO-9205, p. 9, 1960.

8. Willhoit, D. G. and Jones, T. D. Dose and LET distributions in small-animal sized cylinders for a fission neutron spectrum. Radiation Research 44: 263-272, 1970.

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PROJECT GROUP 6

IN VITRO STUDIES ON LONG-TERM EFFECTS OF RADIATION DAMAGE TO HEMATOPOIETIC STEM CELL GROWTH AND DIFFERENTIATION

Principal Investigator: S. R. Weinberg

The objective of this project is to develop technology for tissue culture for the purpose of (a) maintaining hemopoietic stem cells and their progeny for several months, (b) examining the existence and persistence or loss of surface markers on these cells (i.e., antigenicity) with increased length of culture, (c) gaining further insight into the role of a controlled microenvironment on the stem cell proliferation and differentiation into precursor and mature blood cells, and (d) evaluating "cell-to-cell" interactions and "humoral" mechanisms involved in radiation damage and recovery of bone marrow progenitor blood cells.

The liquid culture technique of Dexter and colleagues (1-5) will maintain murine and canine marrow stem cells in vitro for several months. Bone marrow cells

from normal, low-dose, and high-dose irradiated mice and dogs will be morphologically defined, assayed for stem cell activity, and then plated in the liquid cultures, repeatedly fed. At varying times, different stimulants will be added to these cultures of stem cells to trigger proliferation and differentiation. The inability of cells to be maintained for lengthy periods, to respond to stimulants, to differentiate, and to mature will reflect the degree of radiation damage and recovery potential. Continuous monitoring of the previously irradiated stem cells being maintained in vitro for lengthy periods will allow the study of long-term or delayed effects of radiation damage and recovery.

Histological methodology has been used to identify the normal cells plated and the cells recovered from each culture system. Functional hematopoiesis in each system will be assessed by assay of erythrocytic committed stem cell (BFU-E and CFU-E), granulocytic committed stem cell (CFU-C), monocyte-macrophage committed stem cell (M-CFC), and "granuloerythropoietic stem cell" (CFU-G/E).

During 1979 the microplasma clot culture system was established as an assay to monitor erythoid progenitor cells (BFU-E and CFU-E) in the blood cell-forming tissues of experimental animals (i.e., mice and dogs). Preliminary studies were conducted to assess the latent effects of low-dose, total-body, gamma irradiation on the hemopoietic system. Cell suspensions of bone marrow from irradiated mice were plated in the liquid culture systems. No conclusive patterns were observed because different variables in the culture system were also being studied.

REFERENCES

1. Dexter, T. M., Allen, T. D., Lajtha, L. G., Schofield, R., and Lord, B. I. Stimulation of differentiation and proliferation of haemopoietic cells in vitro. Journal of Cellular Physiology 82: 461-474, 1973.

2. Dexter, T. M. and Lajtha, L. G. Proliferation of haemopoietic stem cells in vitro. British Journal of Haematology 28: 525-530, 1974.

3. Dexter, T. M. and Lajtha, L. J. Ontogeny and evolution of normal and malignant hematopoietic cells. Bibliotheca Haematologica 43: 1-5, 1975.

4. Allen, T. D. and Dexter, T. M. Surface morphology and ultrastructure of murine granulocytes and monocytes in long-term liquid culture. Blood Cells 2: 591-606, 1976.

5. Allen, T. D. and Dexter, T. M. Cellular interrelationships during in vitro granulopoiesis. Differentiation 6: 191-194, 1976.

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ULTRASTRUCTURAL ALTERATIONS OF THE EPENDYMAL CELL LINING OF LATERAL VENTRICLES OF PRIMATES WITH EXPERIMENTAL COMMUNICATING HYDROCEPHALUS

Principal Investigator: W. J. Flor, *AFRRI*

Collaborators: A. E. James, Jr., *Vanderbilt University Hospital, Nashville, Tennessee*
J. L. Ribas, *Uniformed Services University of the Health Sciences, Bethesda, Maryland*

Technical Assistance: J. L. Parker, *AFRRI*
W. L. Sickel, *USUHS*

Data from previous studies with our experimental primate model for communicating hydrocephalus (normal pressure) implied that compensatory transependymal pathways for reabsorption of cerebrospinal fluid appear to be established (1,2). In this study we corroborate our physiological data with ultrastructural transmission electron microscopy (TEM) studies and scanning electron microscopy (SEM) studies of lateral ventricular lining and choroid plexus in these animals with chronic hydrocephalus (3).

The rounded and stretched angles of lateral ventricles are the foci of histological alterations in animals about 100 days after Silastic implantation. SEM studies show flattening and stretching of ependymal cells in the dorsomedial angles. Supraependymal cells and axons are more visible. Supraependymal cells are frequent, oblong, phagocytic in appearance, and have multiple branching projections. The dorsolateral angle is most severely affected. Normal ependymal surface on either side transforms abruptly to a region mostly denuded of ependymal cell profiles at the angle. This area is covered with individual stretched or rounded ependymal cells, filamentous processes, some large and many small supraependymal cells, and many smooth small-caliber processes. Correlative TEM supports identification of these elements. Smooth processes of small supraependymal cells contain dispersed microtubules and filaments. Other filamentous processes are of ependymal or astrocytic origin.

TEM also supports the SEM observation that there appears to be ready communication through gaps of up to 1 μm between the ventricle and the parenchymal extracellular fluid. In animals at about 1000 days after implantation, ventricles are significantly enlarged and rounded, and the region of most severe pathology extends considerably farther from the angles. The surface is smoother, since ependymal and astrocytic elements have proliferated and covered over the ventricular lining, but ready communication between ventricular and parenchymal fluid is still present. The surface lacks the small, round supraependymal cells but contains many stellate supraependymal cells of indeterminate origin. These ultrastructural observations are consistent with our hypothesis of compensatory transependymal mechanisms for reabsorption of cerebrospinal fluid in communicating hydrocephalus.

The response (described above) of the ependymal lining of the ventricles to the pathophysiological stresses associated with developing communicating hydrocephalus and also the changes manifest in the supraependymal cell population have led to a study of the supraependymal cells themselves. We are currently investigating the origin, function, and radiosensitivity of these phagocytic cells with both physiological and ultrastructural techniques. Although the mature

phagocyte may be relatively radioresistant, the repopulation of these cells forms pools of more radiosensitive precursors that could be significantly altered post-irradiation, and their availability for participation in posttraumatic tissue responses could be markedly reduced.

REFERENCES

1. James, A. E., Jr., Flor, W. J., Novak, G. R., Strecker, E.-P., Burns, B., and Epstein, M. Experimental hydrocephalus. In: The Ocular and Cerebrospinal Fluids. Bito, L., Davson, H., and Fenstermacher, J. D., eds. The Aberdeen University Press, Ltd., Aberdeen, Scotland, 1977, pp. 435-450.

2. James, A. E., Jr., Novak, G. R., Strecker, E. P., Burns, B. B., Correa-Paz, F., and Flor, W. J. Experimental studies relating to diagnostic imaging in disorders of cerebrospinal fluid circulation. In: Neurobiology of Cerebrospinal Fluid, Vol. 1. Wood, H. J., ed. In press.

3. Flor, W. J., James, A. E., Jr., Ribas, J. L., Parker, J. L., and Sickel, W. L. Ultrastructure of the ependyma in the lateral ventricles of primates with experimental communicating hydrocephalus. Scanning Electron Microscopy 3: 47-54, 1979.

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NEUROBIOLOGY DEPARTMENT

The Neurobiology Department was established in late 1972. Its objective is to describe and elucidate the mechanisms whereby ionizing radiation interferes with nervous system function either directly or by means of substances released by the body during radiation.

The Department is comprised of three Divisions with different approaches to the study of the nervous system. The Radiation Biophysics Division uses conventional electrophysiology techniques to study simple nervous systems such as those from lower vertebrates and invertebrates. The Cellular Neurobiology Division uses tissue culture techniques to study the nerve cells from mammals but in isolation from the animal and growing in an artificial medium. The Neurological Sciences Division, on the other hand, primarily investigates the intact brain of several mammalian species.

The nervous system is relatively resistant to the effects of ionizing radiation. However, at high doses the nervous system is quite dramatically affected, and the primary symptoms and the cause of death at sufficiently high doses are attributable to the direct action of radiation on the brain.

The effects of radiation on nervous tissue fall into three major areas. At relatively low doses, including levels that are used for therapeutic treatment of malignancies in humans, there is a pronounced fatigue following radiation exposure, which may last for many months. This fatigue is often very debilitating, even after exposure to doses that are not fatal, through secondary effects on gastrointestinal or hemopoietic systems. At present the mechanisms underlying fatigue are unknown, and indeed, it is not clear whether this is a central nervous system fatigue or some effects of the radiation at the neuromuscular junction. At exposures of 1000 rads or greater, which are lethal, the primary effects fall into two broad categories and are due to different mechanisms. In a variety of mammals, and particularly in primates, a period of early transient incapacitation (ETI) occurs shortly after exposure. In the monkey, ETI has a latency of 2 to 5 min and lasts for only a matter of minutes. During this transient episode, animals that were trained to perform tasks can function no longer. However, at the end of the period of ETI, the animal recovers function and can continue to perform efficiently until a final deterioration which leads to death. This final deterioration is known as the central nervous system (CNS) syndrome, and is usually accompanied by a very rapid decompensation, coma, and death. The CNS syndrome may occur from a few to 48 hours postexposure, with considerable variation from animal to animal, but with a general dependency on radiation dose.

Although the mechanisms causing these effects of radiation on the central nervous system are not clearly understood, there are experimental leads for each of them. Both fatigue and the CNS syndrome may result from disturbances in regulation of intracellular ionic concentrations. This is suggested by observations indicating that the effect of high doses of radiation on nerve cell membrane potential and resistance is compatible with changes either in membrane permeability or recovery mechanisms. The study of fatigue and the mechanisms of control of intracellular ions are major areas of investigation in the Radiation Biophysics Division.

ETI, by virtue of its latency and its transient nature, appears likely to result from the action of some humoral agent released by whole-body radiation on the central nervous system. One possibility is histamine. It is a very active substance that affects the bronchi of the lungs and blood vessels in the brain and elsewhere in the body, and also has direct effects on neurons.

Previous work in this laboratory has shown that histamine is released upon exposure to radiation. We are continuing to study the effects of histamine and are investigating the presence of receptors for histamine on smooth muscle cells, isolated neurons, and neurons in the central nervous system. A primary aim of the Neurological Division is to study these events in the intact animal.

In whole-animal exposure to radiation, it is impossible to determine the relative radiosensitivity of most cellular elements of the body. This is particularly true for the various cellular elements in the central nervous system, which include nerve cells of different types, glial cells, and the smooth muscle cells of the cerebral blood vessels. A primary aim of the Cellular Neurobiology Division is to develop dividing cell lines of each of these types and to determine their relative sensitivities to radiation.

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MEMBRANE CONDUCTANCE INCREASE IN THE GIANT ABDOMINAL NEURON OF *APLYSIA* INDUCED BY LOW-SODIUM SOLUTIONS

Principal Investigators: J. P. Apland and D. R. Livengood

The effects of low-sodium solutions on membrane conductance in the giant abdominal neuron (R_2) of Aplysia were investigated with electrophysiological methods. We previously showed (1) that perfusion with low-sodium solutions, using choline, glucosamine, mannitol, tetraethanolammonium, and tetramethylammonium as sodium substitutes, caused an increase in membrane conductance in the majority of experiments with all substitutes. This increase was consistently blocked by addition of cobalt to the perfusion solution. Cobalt also blocked anomalous rectification in all cells studied.

Low-sodium solutions characteristically caused a transient hyperpolarization followed by a persistent depolarization (Figure 1). After treatment with ouabain, the cell exhibited a persistent hyperpolarization in low-sodium solutions, indicating that the depolarization seen without ouabain present is due to inactivation of the electrogenic sodium pump as intracellular sodium becomes depleted. When barium was substituted for calcium, results similar to those with cobalt were produced. Anomalous rectification was blocked by barium, and a decrease in membrane conductance in low-sodium perfusion solution was produced where no change in membrane conductance had previously been shown in low-sodium solution. Deletion of potassium from the perfusion solution, or replacement of potassium with rubidium, blocked the increase in membrane conductance previously observed in low-sodium solutions and also blocked anomalous rectification. Cold temperatures, which block anomalous rectification, also blocked the membrane conductance increase in low-sodium solutions. Finally, a lowered extracellular pH blocks the membrane conductance increase in low-sodium solutions. Meech (2) has shown that lowered pH blocks potassium conductance.

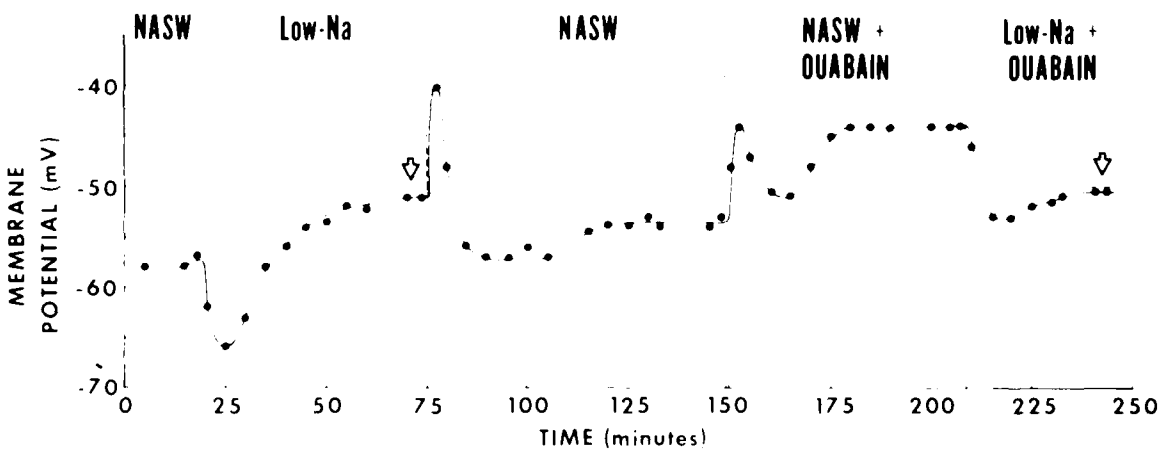

Figure 1. Effect of low-sodium seawater (Low-Na) on membrane potential. Cell was perfused initially with normal artificial seawater (NASW). Perfusion with low-Na (Mg-mannitol-substituted) seawater was begun as indicated, resulting in membrane potential change. Perfusion with NASW was resumed (panel 3). Recovery of membrane potential began. Ouabain was then added to NASW, causing depolarization (panel 4). Perfusion with low-Na and ouabain then began, resulting in a persistent hyperpolarization (panel 5).

These observations with cobalt and barium suggest that calcium is involved in the increase of membrane conductance in low-sodium solutions. The results using potassium-free solutions, rubidium, cold, and reduced pH suggest that the increased membrane conductance is due to potassium. This conclusion is reinforced by the fact that all treatments that abolish anomalous rectification [which is presumed to involve the potassium-conductance system (3)] also block the membrane conductance increase in low-sodium solutions. Reduction of extracellular sodium probably inactivates the mechanism of sodium-calcium exchange, causing intracellular calcium to accumulate and increase potassium conductance. The fact that cobalt and barium (which interact with calcium channels) abolish anomalous rectification suggests that calcium also may be involved in that phenomenon.

REFERENCES

1. Apland, J. P. and Livengood, D. R. Effect of low-sodium solution in the giant abdominal neuron (R_2) of Aplysia. Neuroscience Abstracts 3: 659, 1977.

2. Meech, R. W. Calcium-dependent potassium activation in nervous tissues. Annual Review of Biophysics and Bioengineering 7: 1-118, 1978.

3. Marmon, M. F. The membrane of giant molluscan electrophysiologic properties and the origin of the resting potential. Progress in Neurobiology 5: 169-195, 1975.

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ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERISTICS OF SEROTONIN RECEPTORS ON A NEURONAL SOMATIC CELL HYBRID

Principal Investigators: W. G. Sham and J. F. Fresch

The electrophysiology and pharmacology of serotonin responses on a cell line (TCX11) derived from the fusion of mouse neuroblastoma cells (N18TG2) with mouse sympathetic ganglion cells (1) were studied. Dopamine and serotonin elicit responses of increased depolarizing conductance in these cells (2). The purpose of this study was to characterize the serotonin response and compare the properties of the serotonin responses and the dopamine responses to determine if they are mediated through different receptors.

Iontophoresis of serotonin caused a rapid depolarization of the hybrid cell membrane potential associated with an increase in membrane conductance. The response rapidly desensitized with repeated pulses of serotonin. These same results were obtained when dopamine was iontophoresed, but the cells were 10 to 100 times more sensitive to serotonin than to dopamine. When equal pulses were used, the responses of serotonin and dopamine appeared mediated by different receptors when examined for reversal potentials, cross-desensitization, and antagonist specificity. However, when the iontophoretic pulses were adjusted to give

responses of similar amplitude, different results were obtained. Dopamine and serotonin on the same cell were equal and varied from 0 to +15 mV. Perfusion of the cells with low-sodium medium reduced the serotonin and dopamine responses and shifted the reversal potentials in a similar manner. Possible antagonists were bath applied in concentrations of 10 to 100 μM.

No drug has been found that blocks one response and not the other. The following drugs blocked both responses: (+)-tubocurarine, chlorpromazine, bulbocapnine, phentolamine, metergoline, bromo-LSD methiothepin, cyproheptadine, cinnarserin, and mersalate. Phenolamine, bromo-LSD, and cyproheptadine, applied by blunt micropipette, caused a depolarization and increase in membrane conductance. Absence of this effect during bath application was presumably due to rapid desensitization. Since the drugs that were only bath applied may have had similar agonist effects, we have not characterized the nature of the blockade mediated by any of these drugs.

Pharmacology will be reexamined by establishing dose-response curves for dopamine and serotonin in the presence of varying concentrations of test drugs. In addition, the effects on membrane potential and conductance will be assessed, applying the test drugs by micropipette. The serotonin response of TCX11 appears similar to that on a number of autonomic neurons (3) and on several neuroblastoma clones and neuroblastoma X glioma hybrids (4). It is impossible to know how similar the serotonin receptors are on these various preparations until more detailed pharmacology is performed on all of them.

REFERENCES

1. Greene, L. A., Shain, W., Chalozinitis, A., Breakfield, X., Minna, J., Coon, H. G., and Nirenberg, M. Neuronal properties of hybrid neuroblastoma X sympathetic ganglion cells. Proceedings of the National Academy of Sciences 72: 4923-4927, 1975.

2. Myers, P. R., Livengood, D. R., and Shain, W. Characterization of a depolarizing dopamine response in a vertebrate neuronal somatic cell hybrid. Journal of Cellular Physiology 91: 103-118, 1977.

3. Wallis, D. I. and North, R. A. The action of 5-hydroxytryptamine on single neurons of the rabbit superior cervical ganglion. Neuropharmacology 17: 1023-1028, 1978.

4. MacDermot, J., Haruhiro, H., Wilson, S. P., Matsuzawa, H., Minna, J., and Nirenberg, M. Adenylate cyclase and acetylcholine release regulated by separate serotonin receptors of somatic cell hybrids. Proceedings of the National Academy of Sciences 76: 1135-1139, 1979.

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RESPONSE OF CEREBRAL CIRCULATION TO TOPICAL HISTAMINE

Principal Investigators: A. N. Martins, T. F. Doyle, S. J. Wright, Jr., and B. G. Bass

To study the effect of histamine on brain blood flow and capillary permeability, bilateral parietal craniectomies were made in cats anesthetized with nitrous oxide and ketamine. The dura was removed and solutions of histamine in mock cerebrospinal fluid (in varying concentrations from 10^{-5} M to 10^{-1} M) were irrigated continuously onto the exposed brain. At the same time, local cerebral blood flow was measured polarographically by hydrogen clearance (Table 1).

Table 1. Effect of Topical Histamine on Local Cerebral Blood Flow in Cat

		Histamine Concentration (M)			
	Baseline	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Cerebral Blood Flow*	61 ± 5	65 ± 8	113 ± 20	122 ± 11	124 ± 27
Number of Animals	17	8	11	16	5
p^+		0.048	0.0002	0.0000005	0.004

* ml 100 g⁻¹ min⁻¹ ± SEM

\+ Probability that difference from baseline value would have occurred by chance (paired t test)

Capillary permeability was assessed by determining histamine's effect on the ^{125}I-albumin space of the brain. Electrical activity of the brain was monitored by electrocorticography. Histamine consistently dilated pial blood vessels and produced within 15 min a dose-related local hyperemia that subsided 30-60 min after histamine was removed (Figure 1). Hyperemia was blocked by cimetidine.

Histamine had no appreciable effect on either the blood-brain barrier to albumin or the electrical activity of the cortex. Histamine is pharmacologically capable of participating directly in the acute hyperemic response of the brain's microcirculation to physiologic and pathologic stimuli, but it has little effect on cerebrovascular permeability to protein.

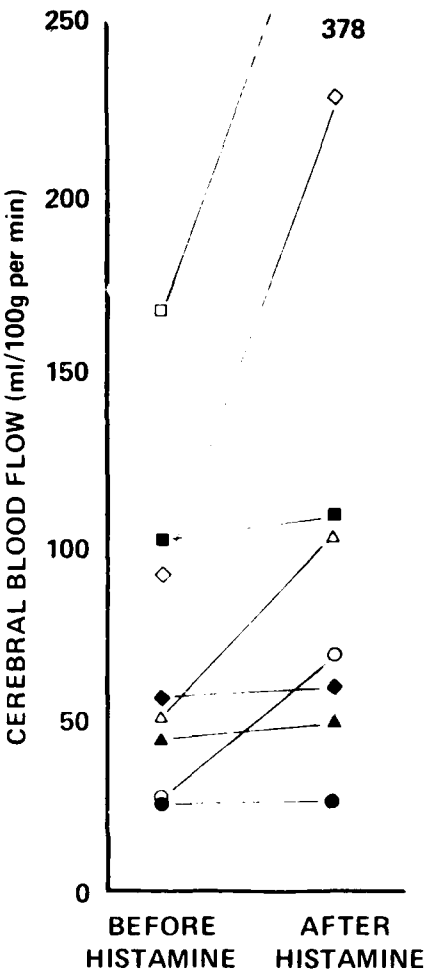

Figure 1. Effect of H_2 antagonist cimetidine on HA-induced hyperemia in 4 cats. Solid symbols are data from hemispheres pretreated with epiarachnoid irrigation of 10^{-2} M cimetidine. Open symbols are from corresponding contralateral untreated control hemispheres. Subsequent irrigation of both hemispheres with 10^{-3} M HA more than doubled mean CBF in control hemispheres while increase of mean CBF in hemispheres exposed to cimetidine was limited to 6%.

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CORTICAL MECHANISMS OF INCAPACITATION SYNDROMES INDUCED BY RADIATION

Principal Investigators: D. G. Braitman and C. R. Auker
Collaborator: R. W. Greene
Technical Assistance: G. Scofield

Exposure to supralethal doses of ionizing radiation results in incapacitation syndromes in experimental animals and in humans. This syndrome is manifest by failure to move and a decrease in responsiveness to sensory stimuli. The aim of these experiments is to investigate the neurogenic basis of this syndrome by examining the effects of various radiation-released neurotransmitters on nerve cells in sensorimotor cortex of the cat. These putative neurotransmitters are released into the brain by mast cells after exposure to ionizing radiation.

Pyramidal tract and nonpyramidal tract neurons were identified in chloralose-anesthetized cats in an initial attempt to characterize the types of cells in this area of the brain. Action potentials were recorded via the center barrel of a five- or seven-barrel micropipette. Each of the other barrels was filled with a putative neurotransmitter. Table 1 shows the percentage of tested pyramidal tract neurons (n = 16) and nonpyramidal tract neurons (n = 36) responding to ionophoretically applied acetylcholine, glutamate, histamine, dopamine, and norepinephrine.

Table 1. Percent Response of Sensorimotor Cortex Neurons to Putative Neurotransmitters

	Acetylcholine		Glutamate		Histamine		Dopamine		Norepinephrine	
Neurons	+	−	+	−	+	−	+	−	+	−
Pyramidal Tract	79	0	100	0	0	80	0	100	0	80
Nonpyramidal Tract	6	36	93	7	36	55	10	45	0	100

+ Excitatory

− Inhibitory

For all transmitters tested, pyramidal tract neurons exhibited a uniform profile of responses, whereas nonpyramidal tract neurons (presumed to be a nonhomogeneous population) showed various responses to the neurotransmitters. These results suggest that the identified populations of output motorneurons from sensorimotor cortex (i.e., pyramidal tract neurons) exhibit a unique profile of responses to application of putative neurotransmitters. Thus, in future experiments, PT neurons may be treated as a homogeneous population of cells.

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LOCALIZATION OF DOPAMINE IN THE GILL OF *APLYSIA*: SOME PHYSIOLOGICAL IMPLICATIONS

Principal Investigators: J. W. Swann, M. G. Pierson, and A. Dahlstrom

The gill of Aplysia contains 3 μg of dopamine per gram of tissue. Physiological experiments using the semi-intact gill preparation led one of us (JWS) to hypothesize that dopamine acts as a neurotransmitter for both motor and modulatory neurons thought to innervate the gill musculature (1). Additional experiments have suggested that L9 neurons of the abdominal ganglion are dopaminergic gill motor neurons (2). In experiments reported here, the distribution of dopamine within the gill is examined anatomically using fluorescence histochemistry for catecholamines. The Hillarp Falck technique was used.

Green fluorescing nerve fibers, with the same emission spectrum as that of dopamine, are distributed on muscle fibers throughout the gill with the notable exception of the bundles of longitudinal muscle fibers of the efferent vessel. Green varicosities, indicative of DA nerve terminals, cover muscle fibers in four areas of the gill: the afferent vessel, the pinnules, the efferent vessel trunklets, and the circular muscles of the efferent vessel. These four areas of the gill will contract when small quantities of dopamine are added to a gill perfusate and, with the exception of the circular muscles of the efferent vessel, they contract upon activation of L9 cells. These observations support the contentions that dopamine is a neuromuscular neurotransmitter in the gill and that L9 cells are dopaminergic.

Contractions of the longitudinal muscle fibers of the efferent vessel are initiated by firing motor neuron LDG_1. These contractions are greatly enhanced by perfusing the gill with dopamine. Since these muscle fibers are not innervated by dopamine-containing nerve fibers, dopaminergic modulation of LDG_1 contractions cannot be mediated physiologically by dopaminergic modulatory neurons as originally proposed. Instead, dopamine modulation of LDG_1 contractions may be humoral in origin. We have found that a major portion of the dopamine content of the gill is located in highly fluorescent structures, which are in close association with the microvasculature of the gill.

REFERENCES

1. Swann, J. W., Sinback, C. N., and Carpenter, D. O. Dopamine-induced muscle contractions and modulation of neuromuscular transmission in Aplysia. Brain Research 157: 167-172, 1978.

2. Swann, J. W., Sinback, C. N., and Carpenter, D. O. Evidence for identified dopamine motor neurons to the gill of Aplysia. Neuroscience, in press.

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EFFECTS OF LITHIUM ON EXCITATORY RESPONSES TO SEROTONIN AND DOPAMINE IN *APLYSIA*

Principal Investigators: A. M. Williamson, T. C. Pellmar, and D. O. Carpenter

Aplysia californica neurons can exhibit similar ionic conductance increases to several neurotransmitters. This has led to the suggestion that there are common ionophores for each class of ionic response (1). If the ionophores mediating the fast sodium conductance increases in response to serotonin and dopamine are identical, then these ionophores should respond similarly to sodium substitutes. Since a different ionophore probably underlies the slow sodium conductance increase to serotonin [A' response of Gerschenfeld and Paupardin-Tritsch (2)], sodium substitutes might act differently on this response.

We tested the effects of substituting lithium for 50% and 100% of the sodium in artificial seawater on the excitatory responses to iontophoretically applied serotonin and dopamine. Preliminary results suggest that the slow excitatory response to serotonin is reduced in amplitude and prolonged by 50% lithium-seawater. There is further reduction of amplitude in 100% lithium-seawater, but the response was not completely abolished. The reduction in amplitude was partially reversible. The fast conductance increases due to serotonin and dopamine were also reduced in amplitude at both lithium concentrations. Those responses were not prolonged by lithium. Normal seawater partially reversed reduction in amplitude of the responses to both neurotransmitters.

The observation that lithium affects the fast excitatory responses to dopamine and to serotonin in similar manners supports the hypothesis that the responses are mediated by the same ionophore. Since lithium acts differently on the slow excitatory response to serotonin, a different ionophore may be involved.

REFERENCES

1. Swann, J. W. and Carpenter, D. O. Organisation of receptors for neurotransmitters on Aplysia neurones. Nature 258: 751-754, 1975.

2. Gerschenfeld, H. M. and Paupardin-Trisch, D. Ionic mechanisms and receptor properties underlying the responses of molluscan neurons to 5-hydroxytryptamine. Journal of Physiology 243: 426, 1974.

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VISCOSITY CHARACTERISTICS OF MIDDLE EAR EFFUSIONS

Principal Investigators: M. L. Wiederhold, *AFRRI*
J. T. Zajtchuk, J. G. Vap, and H. O. DeFries, *Uniformed Services University of the Health Sciences*

Auditory nerve responses to condensation and rarefaction clicks were recorded from the external ear canal of cats using a closed acoustic system. After baseline hearing levels and tympanograms were done for both ears in a series of 15 cats, the eustachian tube on one side was ligated. Then serial recordings were again done on all cats at various intervals. Paracentesis of the middle ear fluid on the ligated side was done when negative pressures or a type B tympanogram was obtained. Viscosity measurements were made and correlated with hearing level, type of tympanogram, and pressure gradient of the middle ear space. In approximately 40% of cats with effusions, glue was obtained between 6 and 104 days post-ligation. These cats all demonstrated flat tympanograms or negative middle ear pressures and a hearing loss. In the remaining cats, the middle ear effusions obtained between 8 and 180 days post-ligation had relatively low viscosities and were associated with flat tympanograms and hearing loss.

These data indicate that viscosity is not clearly related to duration of fluid in the middle ear. Pressure gradients were low in all ears with effusion. Control ears in the same animals showed nearly constant hearing levels and normal pressures. There is poor correlation between hearing loss and the viscosity of effusions. If anything, higher viscosity appears to be associated with milder hearing losses than vice versa.

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BETA ADRENERGIC BLOCKERS REDUCE AUDITORY NERVE AND BRAIN STEM RESPONSES TO ACOUSTIC STIMULI

Principal Investigator: M. L. Wiederhold

Intravenous administration of beta-adrenergic-blocking agents in awake rats has been shown to decrease glucose metabolism in nuclei throughout the auditory central nervous system (1). In an attempt to determine where this effect might be exerted, we have recorded click-evoked electrical responses generated in the cochlea (cochlear microphonic), auditory nerve, and brain stem. All potentials were recorded simultaneously from the external ear canal of barbiturate-anesthetized cats before, during, and after intravenous infusion of propranolol for 1 hour. Dose rates from 0.01 to 1.0 mg/kg/min were used.

The amplitudes of both the auditory nerve response and the brain stem electrical response to clicks approximately 40 dB above threshold were reduced by up to 70% in a dose-dependent manner by propranolol. A half-maximum effect was obtained at approximately 0.3 mg/kg/min. Maximum effects were generally seen at about 10 min after infusion was stopped. Recovery began immediately thereafter but was never complete within 3 hours.

No consistent dose-dependent effects on the cochlear microphonic were seen. As neural responses were reduced during drug infusion, the relationship between amplitudes of auditory nerve response and brain stem electrical response remained the same as when click sound pressure level was reduced before drug administration. As the amplitude of auditory nerve response was reduced by propranolol, its latency increased much less than when sound pressure was lowered, but latency of the brain stem electrical response did increase the same amount as in a click-level series. These findings, coupled with the lack of effect on the cochlear microphonic, suggest that the major site of exertion of effects reported here is at the synapse between hair cell and auditory nerve fiber. Thus there appears to be an adrenergic modulation of either this synapse or the efferent synapses within the cochlea.

REFERENCE

1. Savaki, H. E., Kadekaro, M., Jehle, J., and Sokoloff, L. α- and β-adrenoreceptor blockers have opposite effects on energy metabolism of the central auditory system. Nature 276: 521-523, 1978.

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IONTOPHORESIS OF ACETYLCHOLINE EVOKES A SLOW MUSCARINIC DEPOLARIZATION IN NEURONS OF DISSOCIATED RAT SUPERIOR CERVICAL GANGLION

Principal Investigators: J. E. Freschi and W. G. Shain

Synaptic transmission within the superior cervical ganglion (SCG) has been extensively studied because it is experimentally accessible and contains a diversity of types of synaptic transmission. Further experimental simplification in tissue culture techniques has allowed the useful study of the biochemical basis of neural transmission and its correlation with electrophysiology. Therefore, it is puzzling that the slow synaptic potentials seen in intact animals and isolated whole-organ preparations have not been discovered in primary cultures of mammalian SCG (1).

We have consistently found slow depolarizing potentials in response to iontophoresis of acetylcholine onto neurons dissociated from neonatal rat SCG. With small pulses of acetylcholine, a fast depolarizing response associated with a fall in membrane resistance is evoked. When the fast response reaches saturation with large pulses of acetylcholine, a long-lasting depolarization of delayed onset becomes evident (Figure 1 A,B). The slow acetylcholine response increases in amplitude and duration in a dose-dependent fashion, with a maximum response lasting 30 to 40 sec. The membrane resistance during the slow response either remains unchanged or increases slightly. Curare eliminates the fast acetylcholine potential without affecting the slow response (Figure 1 C). Atropine selectively blocks the slow potential (Figure 1 D). Excitability is increased during the muscarinic action of acetylcholine. This effect is independent of acetylcholine-induced changes in membrane potential. In some cells, hyperpolarizing the

membrane with steady inward current reduces the amplitude of the acetylcholine depolarization but does not reduce the increased excitability effected by acetylcholine. The spike afterpotential is reduced after muscarinic acetylcholine activation, and this effect, too, occurs separately from changes in membrane potential.

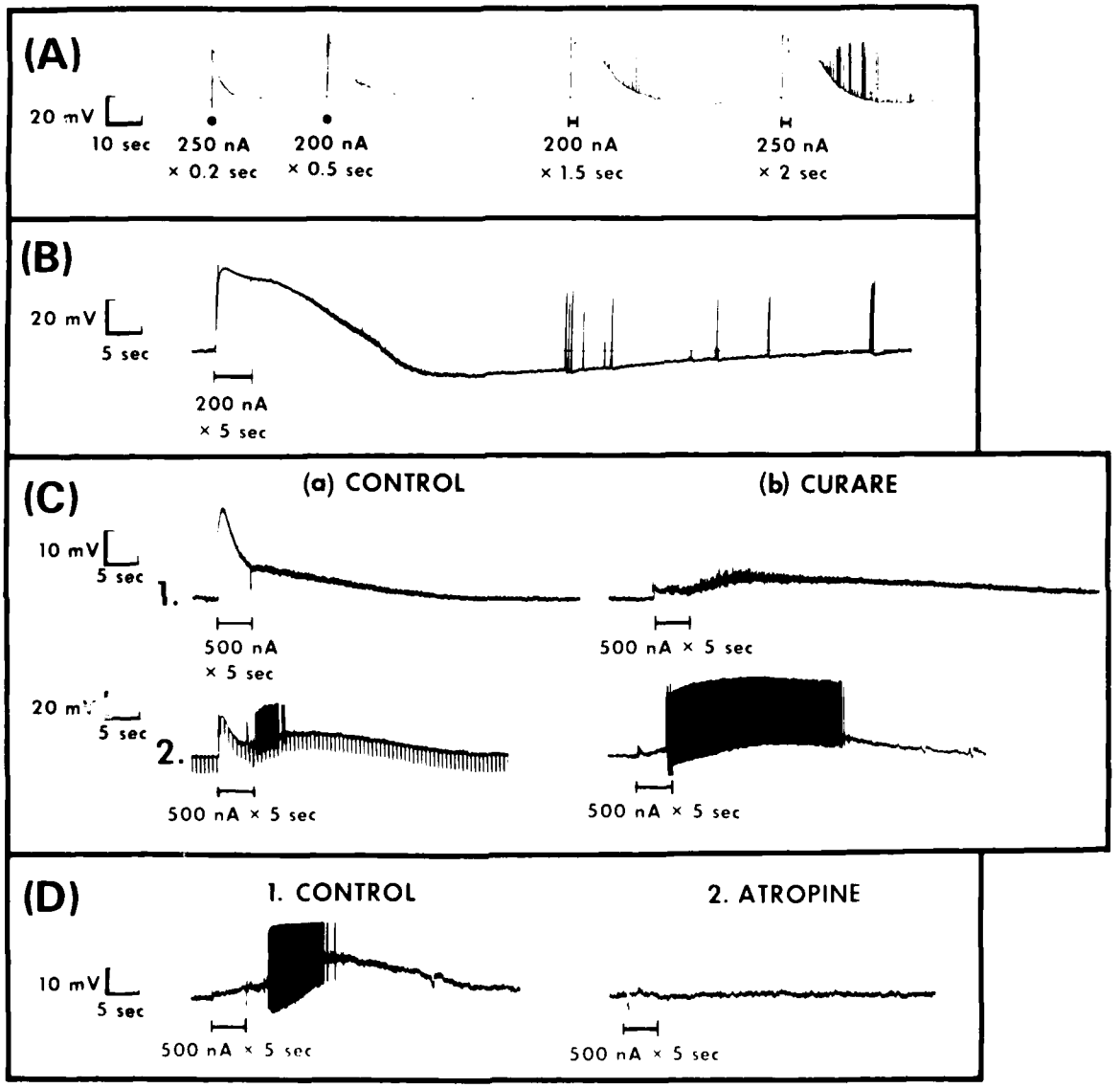

Figure 1. Fast and slow acetylcholine(ACh)-evoked depolarizations and their pharmacological separation. Current X time of ACh iontophoretic pulse indicated below each response. A: responses to increasing pulses of ACh. Resting membrane potential (RMP) −75 mV. B: large biphasic ACh depolarization followed by long hyperpolarization. RMP −70 mV. C: elimination of fast response after 100 μM d-tubocurarine (TC) added to bath. Column a, control response; column b, after TC. C_1 and C_2 represent different cells with RMPs of −60 mV and −70 mV, respectively. During control response of C_2, membrane resistance was being monitored by passing hyperpolarizing constant-current pulses across membrane. D: elimination of slow ACh response after 10 μM atropine added to bath. Fast response had been eliminated by 100 μM TC. RMP −70 mV.

The requirement of a large acetylcholine dose to evoke the slow depolarizing potential suggests that both nicotinic and muscarinic acetylcholine receptors of high affinity (2) must be saturated before lower affinity muscarinic receptors mediating the slow response can be evoked. Our findings offer the opportunity to more easily and unambiguously study the ionic and biochemical mechanisms of muscarinic acetylcholine action.

REFERENCES

1. O'Lague, P. Studies on rat sympathetic neurons developing in cell culture. III. Cholinergic transmission. Developmental Biology 67: 424, 1978.

2. Heilbronn, E. and Bartfai, T. Muscarinic acetylcholine receptor. Progress in Neurobiology 11: 171-188, 1978.

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COCHLEAR MICROPHONIC POTENTIALS FROM INNER AND OUTER HAIR CELLS?

Principal Investigators: M. G. Pierson, *AFRRI*
A. Moller, *University of Pittsburgh School of Medicine*

Generally it is not considered feasible to distinguish between the cochlear microphonic originating from outer hair cells and that originating from inner hair cells in extracellular recordings. The large number of contributing hair cells, their stimulated phase distribution, and the finding that there is a 40-dB difference in their sensitivities has promoted this notion. In the present study it is reported that within a narrow window defined by intensity and frequency, two types of cochlear microphonic can be discerned simultaneously. Specifically, within a quarter-octave band, one-fifth to one-half octave above the best frequency of a differential electrode pair, the cochlear microphonic intensity function displays a feature suggesting a dual origin: it has two maxima separated by a cancellation notch at about 70 dB sound pressure level.

Stimuli used in the present study consisted of 120-msec pure-tone pulses with linear rates of rise and fall, obtained by using analogue multipliers and trapezoidal control signals. The rise-fall times were generally 40 msec, the on time was less than 120 msec, and the period was 1 sec. The cochlear microphonic was band passed with a one-third octave-band filter tuned according to the stimulating frequency. Amplitude measurements were obtained by the full wave rectification of the cochlear microphonic, followed by a low pass filtering (470 Hz) and subsequent averaging. In experiments testing the influence of basilar membrane position, cochlear microphonic responses were processed as above except that the stimuli consisted of continuous pure tones. Basilar membrane position was modulated sinusoidally by presenting a 100-Hz signal together with the continuous high-frequency probe signal. Cycle histograms of the relative amplitude of the cochlear microphonic were then generated when the averager was triggered by the 100-Hz signal.

On the basis of these experiments, it is proposed that the cochlear microphonic originating from outer hair cells has a lower threshold, a smaller dynamic range, is more subject to acoustic fatigue, and is more labile due to metabolic impairment (hypoxia) than the cochlear microphonic originating from inner hair cells.

The magnitude of this type of cochlear microphonic is enhanced by a movement of the basilar membrane toward the scala tympani. On the other hand, the cochlear microphonic presumed to arise from inner hair cells is insensitive, has a vast linear operating range, and is almost unassailable by intense acoustic exposure or by hypoxia. This latter type of cochlear microphonic is enhanced by movement of the basilar membrane toward the scala vestibuli.

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LIGHT-GENERATED HEAT IN THE EYE: ROLE OF THE CHOROIDAL CIRCULATION AS A HEAT SINK

Principal Investigators: L. M. Parver, *Georgetown University*
C. R. Auker and D. O. Carpenter, *AFRRI*

The choroidal circulation accounts for 85% of the total ocular blood flow. Compared to circulation in the renal cortex, the choroid has four times the volume of blood flow per 100 grams of tissue. The question arises as to whether the high-flow choroidal circulation is functioning solely to supply nutrients to the outer avascular retinal layers. The exceptionally high oxygen content of venous choroidal blood, about 95% of that found in arterial blood, suggests some other role. One such role might be the dissipation of heat generated by the absorption of focused light in the outer retinal layers and the retinal pigment epithelium.

To study this hypothesis, temperature measurements were taken from the macula of the cynomolgus monkey eye. A thermistor probe was inserted through the pars plana and positioned in the macula under direct opthalmoscopic control. The animals were exposed to either of two light environments: a dimly lit room or a lamp delivering 1.09 mW/cm^2 at the corneal surface. Temperature measurements were taken under these two light conditions while choroidal blood flow was decreased by raising intraocular pressure.

Raising the intraocular pressure with the animals exposed to the illumination in a dimly lit room produced a decrease in temperature recorded in the macula. In comparison, those animals exposed to a 1.09-mW/cm^2 light source showed an increase in macular temperature when the intraocular pressure was elevated. Temperature measurements were also taken from a peripheral retinal site. Increasing the intraocular pressure here produced a decrease in temperature irrespective of the light source. The decrease in temperature was slightly less marked with the 1.09-mW/cm^2 light source, but it ran a course parallel with the changes observed with only background illumination.

We conclude that in low-light environments, the choroidal circulation acts as a heat source, maintaining a constant-temperature environment for the retina and the retinal pigment epithelium. In high-light environments, the choroidal vasculature switches to a "heat sink," dissipating the heat generated by the absorption of focused light at the macula.

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RELEASE OF β-ADRENERGIC AGONIST FROM SYMPATHETIC NEURONS IN CO-CULTURE WITH PINEAL CELLS

Principal Investigators: A. Parfitt and W. G. Shain

Co-cultures of pineal cells and sympathetic neurons were prepared from dissociated pineal glands and superior cervical ganglia dissected from 2-day-old rats. Routinely 300,000 pineal cells were plated with 30,000 neurons. Cultures were maintained in Ham's F12 containing 10% fetal calf serum at 36.5°C in an atmosphere of 4% CO_2/96% air. Medium was changed every third day. Experiments were done 5 to 8 days after culture preparation. Test compounds were incubated with the cultures for 6 hours. To monitor spontaneous release of β-agonist, co-cultures were incubated with compounds that block the reuptake of norepinephrine by nerve endings.

Both desmethylimipramine (DMI) (10^{-5} M) and cocaine (10^{-6} to 10^{-4} M) caused an increase in acetyl CoA:serotonin N-acetyltransferase (NAT, EC 2.3.1.5) activity in the pineal cells. Neither DMI nor cocaine affected basal or isoproterenol-stimulated NAT activity in pineal cells cultured alone. The DMI-stimulated or cocaine-stimulated increase in NAT activity in co-cultures was not affected by simultaneous treatment with tetrodotoxin (10^{-7} M). This concentration of tetrodotoxin was sufficient to block electrically initiated action potentials in the cultured neurons. The increase in NAT activity after treatment with DMI and cocaine varied between preparations, probably as a result of variations in the numbers of neurons in the cultures prepared on different days. This increase in NAT activity was never less than 50% and often approached 80% of the activity elicited by a maximally effective concentration of isoproterenol (10^{-8} M).

In order to monitor evoked release from neurons, co-cultures were incubated with batrachatoxin (10^{-7} M), which is reported to stabilize voltage-dependent sodium channels in the open configuration, thus depolarizing the neurons. Batrachatoxin treatment elicited an increase in NAT activity, which was blocked by simultaneous application of tetrodotoxin, but was only slightly diminished by treatment with 4×10^{-2} M magnesium chloride. Cobalt was tested but was found to kill both the neurons and the pineal cells. Neither tetrodotoxin nor batrachatoxin affected basal or isoproterenol-stimulated NAT activity in pineal cells. Culture medium taken from the batrachatoxin- or DMI-treated co-cultures initiated an increase in NAT activity when added to cultures of pineal cells alone. This enabled us to

bioassay β-adrenergic agonist released by the neurons in co-culture. Calculations on data from a representative experiment indicated that an amount of β-agonist equivalent to 6.7×10^{-12} moles of norepinephrine was released from each neuron during a 6-hour treatment with batrachatoxin.

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POTENTIATION OF SPONTANEOUS TRANSMITTER RELEASE AT THE NEUROMUSCULAR JUNCTION BY COBALT CHLORIDE

Principal Investigators: L. M. Masukawa and D. R. Livengood

Miniature endplate potential (mepp) frequency of the frog neuromuscular junction will vary due to changes in concentration of internal ionizing calcium. Strontium and barium are able to support release when applied externally. Many more divalent cations either block calcium channels and block release or have no effect on spontaneous release at concentrations that block calcium conductance.

A low concentration (0.1 μg/ml) of the specific neurotoxin beta-bungarotoxin (22,000 daltons) was used to raise the mepp frequency to about 30 times that of nontreated levels (0.2/sec) in normal Ringer's solution. A concentration of cobalt (2 mM) that blocked evoked release, had very little effect on mepp frequency under control conditions. However, after toxic pretreatment, replacement of calcium with 2 mM cobalt produced a dramatic increase in mepp frequency (500%) (Figure 1). This increase cannot be explained merely by an increase in the phospholipase activity of the toxin by cobalt. The increase seen with cobalt Ringer's solution appears to be due to the toxin's forming new pathways into the presynaptic terminal.

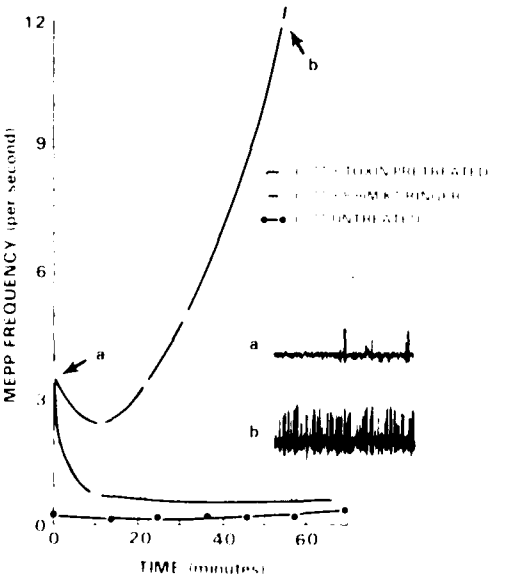

Figure 1. Effects of cobalt chloride (2 mM) on miniature endplate potential (mepp) frequency under various experimental conditions. Cobalt negligibly affected mepp frequency of a muscle that had not been exposed to β-bungarotoxin (●). After toxin treatment (0.2 μg/ml), cobalt first decreased mepp frequency and then increased frequency to more than three times that under posttoxin conditions in 1.8 mM calcium Ringer's solution (○). By comparison, cobalt decreased mepp frequency only in fiber from nontoxin-treated muscle exposed to 8 mM potassium chloride during same period (□). Potassium (8 mM) elevated mepp frequency to approximately that of toxin-treated muscles in control Ringer's. Recording chamber was perfused with cobalt chloride Ringer's starting at time zero in all cases. Insert illustrates chart recordings of miniature endplate potentials of toxin-treated fiber before (a) and after (b) switching to 2 mM cobalt chloride Ringer's.

Therefore cobalt, a substance that normally blocks transmission, can potentiate spontaneous release if allowed to enter the terminal and act on internal sites. Similar effects have been seen by Kita and Van der Kloot (1) with cobalt, using depolarization and the ionophore X357A.

REFERENCE

1. Kita, H. and Van der Kloot, W. Effects of the ionophore X537A on acetylcholine release at the frog neuromuscular junction. Journal of Physiology 259: 177-198, 1976.

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NUCLEAR SCIENCES DEPARTMENT

The Nuclear Sciences Department is composed of two Divisions: the Nuclear Biology Division and the Radiological Physics Division.

In Fiscal Year 1979, the Nuclear Biology Division used radionuclides in both in vivo and in vitro animal models. The in vivo methods provided data noninvasively on internal animal physiology. For more precise quantitative characterizations of organ distribution or bioassay, we used in vitro counting techniques involving beta-emitting and gamma-emitting radionuclides.

During this time, the Nuclear Biology Division had seven active research units relating to several areas of military biomedical concern.

(a) Pulmonary function studies continued for a second year on a series of dogs exposed to neutron or gamma radiation for the determination of longer term effects on canine lung.

(b) We investigated the healing of traumatic fractures that had been surgically induced and also the acceptance of bone grafts, as determined by imaging methods.

(c) We further clarified the distribution of indium-111 using animal models for lymphoscintigraphic applications and for scanning of bone marrow.

(d) We performed xenon-133 ventilation studies using a canine model to observe the retention times of noble gases in several body compartments in vivo.

(e) The work unit on effects of irradiation in the task of handling radiopharmaceuticals took a new direction, in which we evaluated the effects of an antiemetic after irradiation with cobalt-60 gamma or fission spectrum (modified) neutrons.

(f) We revised the diagnostic model for pulmonary embolism to use technetium-99m-sulfur colloid rather than xenon-133 ventilation.

(g) Effort was greatly increased in the evaluation of cardiac localizing radiopharmaceuticals in order to establish appropriate procedures for an extensive series of studies on cardiovascular function postirradiation.

The experimental models and radionuclide techniques in these studies are being developed for application in a series of irradiations using a wider variety of animal models. In Fiscal Year 1980, all these work units will be completed, and the gained experience will be applied to formulating a new research program specifically aligned with the goals of the Institute's 5-year plan. During Fiscal Year 1979, the Division's nuclear imaging equipment was completely rebuilt or replaced, and physics testing for acceptance was performed.

The Radiological Physics Division provides dosimetry support for all radiation sources at AFRRI. Although its function is primarily supportive in nature, it is a highly scientific function requiring extensive research on in-house dosimetry. Its main areas of study are (a) measurements of tissue-to-air ratios; conversion of air doses to tissue doses using tissue-equivalent phantoms; (b) field mapping and measurement of dose distribution; and (c) new dosimetry systems for adaptation of the AFRRI program.

DIAGNOSTIC MODEL FOR PULMONARY EMBOLISM

Principal Investigators: F. Vieras, E. L. Barron, M. P. Grissom, and J. P. Jacobus

Problems concerning the specificity of ventilation-perfusion studies in the diagnosis of pulmonary embolism as well as recognition of the fact that the major cause of pulmonary embolism is deep vein thrombosis have led to the extension of this study into a model for deep vein thrombosis.

The potential usefulness of technetium-99m-sulfur colloid as a radiopharmaceutical for the detection of a thrombus was evaluated in an animal model of deep vein thrombosis. One hour after injection of technetium-99m-sulfur colloid and 24 hours after induction of thrombus formation, a mean thrombus-to-blood uptake ratio of 11.38 was obtained in 12 beagles (see Table 1). This ratio is high enough to allow external detection.

Table 1. Thrombus Accumulation of ^{99m}Tc-Sulfur Colloid

Dog No.	Thrombus Weight (mg)	Uptake Ratios: Thrombus-to-Blood	Uptake Ratios: Endothelial Plaque-to-Blood	Uptake Ratios: Control Vein-to-Blood
1	187	20.16	2.52	0.50
2	134	11.24	3.48	1.64
3	50	9.53	1.47	1.89
4	166	14.35	3.08	1.14
5	236	9.54	3.24	0.43
6	50	11.83	0.85	1.77
7	205	8.20	3.89	1.34
8	141	8.90	2.24	0.49
9	33	31.90	2.46	1.04
10	115	1.70	1.08	0.33
11	31	5.42	1.76	0.39
12	204	3.76	6.04	1.37
Mean ± S.E.	129 ± 21	11.38 ± 2.33	2.68 ± 0.41	1.03 ± 0.17

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PULMONARY OXYGEN TOXICITY

Principal Investigators: K. G. Mendenhall and F. Vieras, *AFRRI*
P. K. Weathersby, E. E. P. Barnard, L. D. Homer, and
S. Survanshi, *Naval Medical Research Institute*

The kinetics of the uptake and elimination of xenon gas in seven anesthetized dogs was studied by simultaneous external recording of gas concentrations in several thousand anatomic sites during 7-hour experiments.

The data were analyzed by a method of extracting moments of the distribution of gas residence times. Mean residence times (first moment) varied by more than a factor of 50 within a single animal. The fastest exchange was in the lungs (under 2 min). Progressively slower exchange occurred in the brain, spinal cord, ears, peripheral joints, and shoulder (over 2 hours). Variance of residence time (second moment) was found to approximate four times the mean residence time squared. This ratio was nearly the same throughout the body. Indications of unexpectedly high xenon solubility in the ear and joint regions were also found. Figure 1 shows the near residence times for several regions.

Region	Samples	Animals
Eye	11	5
Brain	8	5
Spine	7	4
Shoulder	8	4
Knee	4	4
Long Bone	13	6
Wrist	9	5

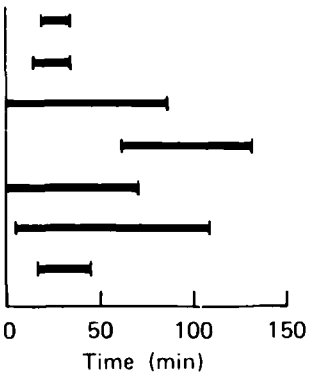

Figure 1. Mean residence times for all experiments

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EVALUATION OF HEALING OF TRAUMATIC BONE FRACTURES AND BONE GRAFTS

Principal Investigators: R. G. Triplett and J. F. Kelley, *Naval Medical Research Institute*
K. G. Mendenhall and F. Vieras, *AFRRI*

In an attempt to develop improved methods of evaluating the fate of bone grafts, we have investigated radionuclide bone imaging using a gamma camera interfaced to a digital computer. A standardized grafting technique was used for removal and replacement of the jaw bone in beagle dogs divided into two groups.

All allogeneic grafts were clinically successful, and continuity of the mandibles was restored. The mean activity ratio (MAR) in the host increased 5.90 ± 1.87 at 2 weeks and then fell to 3.89 ± 0.67 at 8 weeks. In contrast, the MAR in the graft gradually increased to 4.40 ± 1.60 at 6 weeks and remained at approximately this level (shown in Table 1). The heterografts (from sheep) did not have MAR's in the two regions that were similar until 6 weeks (Table 2). As determined by the Wilcoxon ranked sum test, the statistically significant difference in activity between the two regions occurred at the 2-week interval.

Table 1. Comparison of Mean Activity Ratios for Allogeneic Grafts

Image Interval (Weeks)	Host (Mean ± SD)	Graft (Mean ± SD)
1	4.35 ± 1.23	2.75 ± 0.79
2	5.90 ± 1.87	3.14 ± 0.69
4	4.65 ± 1.38	3.61 ± 1.07
6	4.17 ± 0.61	4.40 ± 1.60
8	3.89 ± 0.67	4.11 ± 0.82

Table 2. Comparison of Mean Activity Ratios for Heterogeneic Grafts

Image Interval (Weeks)	Host (Mean ± SD)	Graft (Mean ± SD)
1	4.06 ± 1.38	2.64 ± 0.55
2	4.14 ± 1.25	2.30 ± 0.84
4	5.56 ± 2.53	3.44 ± 1.42
6	4.83 ± 2.70	3.27 ± 1.35
8	4.73 ± 2.20	3.24 ± 0.9[illegible]

EFFECTS OF IRRADIATION ON PULMONARY FUNCTION

Principal Investigators: E. W. Bradley, R. E. McWhorter, W. W. Olmsted, M. P. Fisher, and C. C. Rogers, *George Washington University*
P. O. Alderson, *Columbia-Presbyterian Hospital, New York, New York*
F. Vieras and K. G. Mendenhall, *AFRRI*

Thirty-nine adult, male beagles received either fast neutron or photon irradiation to the right thorax; control dogs were not irradiated. Twenty-four dogs received fast neutrons with a mean energy of 15 MeV to total doses of 1000, 1500, 2250, or 3375 rads delivered in four fractions per week for 6 weeks. Fifteen dogs received total doses of 3000, 4500, or 6750 rads of photons (cobalt-60) in the same fractionation pattern.

Radionuclide evaluations of pulmonary function were performed preirradiation and every 3 months postirradiation for 2 years. These included (a) radioaerosol deposition of technetium-99m-phytate, an insoluble radiocolloid; (b) xenon-133 ventilation studies; and (c) technetium-99m-macroaggregated albumin perfusion images. A chest radiograph taken before each radionuclide evaluation was measured for density changes. Mechanical properties of pulmonary function were studied preirradiation and at 3-month intervals postirradiation for 1 year.

Values for the relative biological effectiveness (RBE) of fast neutrons in producing changes in these parameters were obtained by plotting the changes from preirradiation values in the right lung as a function of the total dose. The RBE for neutron damage to normal lung tissue was always greater than 4 in the clinical dose range of 4000-6000 rads of photons regardless of the parameter used to generate the value (see Table 1).

Table 1. Values of Neutron Relative Biological Effectiveness for Lung Damage

Photon Dose (Total Rads)	Time Postirradiation: 12 Months	18 Months	24 Months
	Relative Distribution of Xenon-133 at Equilibrium:		
4000	6.1 ± 17.0	3.4 ± 0.7	>4.0
6000	8.1 ± 5.1	4.1 ± 1.1	4.9 ± 1.6
	Relative Deposition of Aerosol:		
4000	>4	>4	>4
6000	>6	>6	>6
	Relative Distribution of Perfusion:		
4000	8.9 ± 2.5	6.2 ± 1.6	10.8 ± 4.7
6000	4.8 ± 0.3	5.6 ± 0.5	5.3 ± 0.4

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LOCALIZATION OF INDIUM-111 IN BONE MARROW

Principal Investigators: F. Vieras and M. P. Grissom, *AFRRI*
R. F. Hamilton, *Veterans Administration Medical Center, New York, New York*
R. F. Kiepffer, *National Naval Medical Center*
J. F. Vandergrift, *University of Arkansas, Fayetteville, Arkansas*

The feasibility of using indium-111 colloid for imaging lymph nodes was first demonstrated in dogs by Goodwin and Finston several years ago. However, to the best of our knowledge, there have been no subsequent reports regarding lymphatic scintigraphy with indium-111 colloid in experimental animals.

Indium-111 colloid was evaluated both in vitro using a rat model (Table 1) and in vivo using a canine model in order to image the para-aortic lymph nodes. It was found that despite good distribution in rodents, the in vivo performance varied widely from animal to animal.

Table 1. Tissue Distribution in Rats After Unilateral Pedal Injection of Indium-111 Colloid

Tissue	Percent Injected Dose in Total Organ
Whole Blood	0.26 ± 0.09 *
Bone Marrow	0.80 ± 0.27 *
Spleen	0.66 ± 0.32
Liver	9.06 ± 2.46
Lung	0.79 ± 0.22
Kidney	1.10 ± 0.44
Popliteal Nodes	2.39 ± 0.74
Lumbar Nodes	0.60 ± 0.23
Foot	75.62 ± 9.74

* Percent injected dose per gram that is equivalent to about 4% in whole blood and 5% in bone marrow

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EVALUATION OF MYOCARDIAL RADIOPHARMACEUTICALS

Principal Investigators: F. Vieras and M. P. Grissom, *AFRRI*
R. E. Gibson, W. C. Eckelman, and R. C. Reba, *George Washington University*

Distribution of $|^3H|$ quinuclidinyl benzilate and its methiodide salt was determined in rat, guinea pig (Figure 1), and rabbit. Accumulation in the myocardium of up to 2% of the injected dose per gram of tissue was obtained with both compounds, providing heart-to-blood ratios of about 30 and heart-to-lung ratios of about 4. Accumulation in the heart was blocked (>95%) by preinjection of atropine.

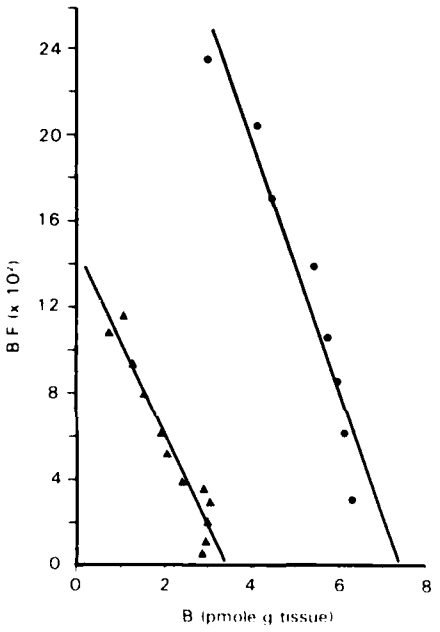

Figure 1. Scatchard plot of $[^3H]$ quinuclidinyl benzilate (●) and $[^3H]$ MQNB (▲) binding to the heavy membrane fraction of guinea pig ventricular muscle

Distribution of tritium in rabbit heart corresponds to the densities of muscarinic receptors determined in vitro. Calculation of the theoretical maximum of the bound-to-free ratio, based on in vitro equilibrium binding isotherms, provides ratios in reasonable agreement with the experimental results.

Because of the high accumulation in the heart with low serum concentration, we conclude that the methiodide salt of quinuclidinyl benzilate is the ideal parent structure for design of a receptor-binding, gamma-emitting radiopharmaceutical for imaging the myocardium.

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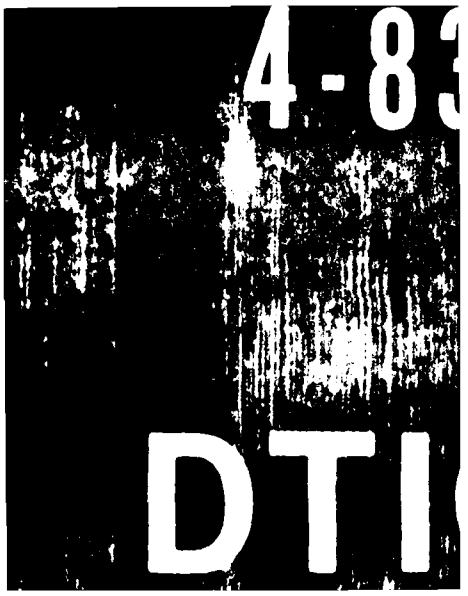
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